ORIGINAL ARTICLE

Chromosomal damage and polymorphisms of DNA repair genes *XRCC1* and *XRCC3* in workers exposed to chromium

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Abstract

OBJECTIVES: Welders, chronically exposed to hexavalent chromium, may exhibit disturbances in chromosomal integrity. Our study investigates chromosomal damage related to chromium exposure, considering the role of polymorphisms in relevant DNA repair genes.

METHODS: 39 male welders exposed to chromium for 10.2±1.67 years and 31 male controls were assayed for structural chromosomal aberrations (conventional cytogenetic analysis). DNA repair gene polymorphisms were determined by Real-Time allelic discrimination assay.

RESULTS: Total chromosomal aberrations were moderately, but statistically not significant, higher in exposed individuals (1.96%) than in controls (1.55%). Chromosomal type breaks were almost two-fold higher in exposed than in control individuals. The highest frequency of total chromosomal aberrations was recorded in individuals with homozygous variant Gln/Gln cariers (2.14%) in *XRCC1** Arg-399Gln and the lowest in those with the wild-type Arg/Arg cariers (1.33%). Polymorphisms in *XRCC3* gene did not modulate the frequencies of CAs, CTAs and CSAs.

CONCLUSIONS: Understanding the effects of chromium on chromosomal integrity in relation to individual susceptibility may be a basis for preventive measures in working process.

INTRODUCTION

Welders are regularly exposed to higher levels of chromium at the workplace in comparison with the non-exposed individuals; respiratory tract being the major route of exposure. Based on *in vitro* and animal data as well as on epidemiological and cytogenetic studies in humans (IARC, 1990), IARC has classified hexavalent chromium as a carcinogen of the group I. In cells, chromium induces formation of reactive intermediates, resulting in enhanced oxidative stress (Leonard *et al*., 2004). The reduction of Cr(VI) to Cr(V) is required for the induction of DNA damage and mutations (Quievryn *et al*., 2002). Genotoxic effects of chromium are predominantly represented by formations of DNA strand breaks (Hodges *et al*., 2001). Human organism has developed many protective mechanisms against DNA damage. Above protection may be executed by the xenobiotics biotransformation (Zhang *et al*., 2006) and by DNA damage repair (Wood *et al*., 2001).

Cytogenetic analysis in peripheral lymphocytes is traditionally used to evaluate an exposure to clastogens (Albertini *et al*., 2003) and cytogenetic parameters may provide information on structural chromosomal damage, often predictive for increased cancer risk (Fučič *et al*., 2007).

Genetic polymorphisms in DNA repair genes may affect DNA repair capacity and thus modify individual response to genotoxic agents and thereby increase cancer risk (De Boer, 2004; Norppa, 2004a; Norppa, 2004b). Association between genetic polymorphisms and intermediary markers may provide useful information on the modulating effects of individual susceptibility (Vodicka *et al*., 2007).

In the present study, we investigated the potential link between the frequences of CAs and genetic polymorphisms in DNA repair genes *XRCC1** Arg399Gln involved in BER and *XRCC3** Thr241Met involved in recombination repair and in maintaining chromosomal stability.

SUBJECTS AND METHODS

Characteristics of subjects

The study was performed on 39 healthy male chromium-exposed welders and 31 healthy male control individuals that were not exposed to any known mutagens or other heavy metals. The detailed population characteristics and smoking habit are given in Table 1. Each person included into the study filled in the questionnaire on life style and tentative medication and signed informed consent. Ethical approval, based on the Declaration of Helsinki, was provided by the Institute of Medical Biology, Jessenius Faculty of Medicine in Martin, Slovak Republic.

Determination of chromium in the blood

Chromium analysis was made, using the atomic absorption spectrophotometer AAS Varian Spectr. AA 30 P. All samples were assessed in duplicate or in triplicate, if significant differences occurred.

Determination of chromosomal aberrations

CAs were determined in two parallel cultures of peripheral blood lymphocytes (PBL). Slides were coded and 100 metaphases per individual were scored for the presence of total number of CAs as well as for chromosome-type aberrations (CSA), and chromatid-type aberrations (CTA) (Bavorova & Ocadlikova, 1989).

DNA repair polymorphisms

Single nucleotide polymorphisms in genes encoding DNA repair enzymes were determined by Taqman SNP genotyping assays ("Assay-by-Demand", Applied Biosystems, Foster City, USA) using Real-Time allelic discrimination on AB 7500 equipment (Applied Biosystems, Foster City, USA) (Vodicka *et al*., 2006).

Statistical calculations

Statistical calculations were performed using Statgraphics, version 7, LEAD Technologies, Inc., USA. For testing significant differences between groups, the non-parametrical Mann-Whitney *U*-test was applied. When more than two parameters were evaluated, Kruskal–Wallis test was applied. Spearman coefficient was used to estimate the correlation between parameters.

Table 1. Characteristics of investigated population

	Exposed group (n=39)	**Control group** (n=31)
Age±SD (years)	38.6±2.07	37.7±2.71
Employment±SD (years)	10.2±1.66	–
Smoking S/NS	21/18	19/12

All individuals included into the study were males.
S - smokers, NS - non-smokers, SD - standard deviation

Table 2. Frequencies of structural chromosomal aberrations and chromium level in the blood of the exposed and the control groups

	Number of individuals (n)	**CAs±SD** (%)	**CTA±SD** (%)	**CSA±SD** (%)	**Total Cr ± SD in the blood** µmol.l-1
Exposed group	39	1.96±1.08	0.92±0.96	1.03±1.09	0.07±0.04
Control group	31	1.55±0.68	1.00±0.63	0.55±0.68	0.03±0.007

CA - chromosomal aberrations (total), CTA chromatid-type aberrations, CSA - chromosome-type aberrations,
*($p<0.05$), SD - standard deviation

RESULTS

In general, the chromosomal damage in the exposed group consisted predominantly of CSAs (1.03%), whereas in the controls the prevailing damage was represented by CTAs (1.00%). Frequencies of total CAs, CTAs and CSAs of exposed and control groups are presented in Table 2. While frequencies of CTAs were fairly similar in both the control (1.00%) and the exposed groups (0.92%), the frequency of more serious CSAs were higher in the exposed group (1.03 %) than in the control group (0.55%).

Significantly lower CAs were detected in individuals with homozygous wild-type polymorphism in *XRCC1** Arg399Gln gene (Tab.3) as compared to those with heterozygous and homozygous variant genotypes (1.33%, 1.80% and 2.14%, respectively; P<0.05). The same tendency was observed in all subgroups after the stratification, being most pronounced in the control group (1.18%, 1.57% and 2.16% respectively; P<0.01).

Frequencies of CTA were the highest in individuals with variant-type Gln/Gln genotype in *XRCC1** Arg-399Gln then in those with heterozygous Arg/Gln and wild-type Arg/Arg genotypes (1.43%, 1.00% and 0.71% respectively).

Polymorphisms in *XRCC3* gene did not modulate the frequencies of CAs, CTAs and CSAs. We did not find any correlation between chromium level in the blood and chromosomal aberrations

DISCUSSION

Our previous works (Halašová *et al*., 2001; Halašová *et al*., 2005) as well as many other epidemiological studies (Davies *et al*., 1991; Gibb *et al*., 2000) showed that workers in ferrochromium industry have an excess risk for chromosomal injury and lung cancer. There is no satisfying explanation for chromium carcinogenicity. Many studies relate this ability to evidenced genotoxicity of chromium (Mitelman *et al*., 1997; Bonassi *et al*., 2000; Hagmar *et al*., 2004)). One of genotoxic damages can be caused by reactive oxygen species that are formed during chromium activation (Wetterhahn *et al*., 1989). Reparation of DNA errors caused by reactive oxygen species plays the crucial role in the prevention of stable DNA damages. Therefore the aim of this study was to conduct a biomonitoring study in welders by employing CAs analysis in peripheral blood lymphocytes as a marker of genotoxic effect in relation to genetic polymorphisms of genes *XRCC1* and *XRCC3* encoding principal DNA repair enzymes as biomarkers of individual susceptibility to mutagenesis and carcinogenesis.

Several studies demonstrate that CAs in PBL are useful and sensitive end-points for studying genetic effects caused by genotoxic agents in populations chronically exposed to various xenobiotics (De Boer, 2002; Norppa 2004a; Norppa, 2004b; Vodicka *et al*., 2004; Vodička *et al*., 2007).

In the current study we disclosed only minor difference in CAs frequencies between the exposed and the control groups. Significant differences in CSAs, considered as genetically more serious damages, suggest formation of DNA double-strand breaks (DBS) after exposure to mutagenic agent, including hexavalent chromium (Pfeiffer *et al*., 2000). All pathways of DSB repair have the potential to induce CA of the exchange type and may explain the origin of chromosome – as well as chromatid – type CA, depending on the cell cycle stage in which DSB are induced. It may occur, if DSB are not repaired or repaired incompletely (Bryant, 1998).

Many authors informed about relationship between single gene polymorphism in *XRCC3* Met241Thr and frequency of micronuclei, chromosomal aberrations and carcinogenesis (Mateuca *et al*. 2008; Stackpole *et al*., 2007; Skjelbred *et al*., 2006; Pérez-Cadahía *et al*., 2008; Yen *et al*., 2008; Zhang *et al*., 2007). Our results showed no significant difference in frequency of CAs, CTA and CSA between different polymorphisms of *XRCC3* gene. This is in the contradiction with mentioned findings. We need to enlarge our groups and provide other cytogenetic analyses.

On the other hand we found that frequencies of CSA in individuals with wild-type *Arg/Arg XRCC1* genotype

Table 3. Frequencies of total chromosomal aberrations (CAs), chromatide-type (CTA) and chromosome-type (CSA) aberrations stratified according to the polymorphisms in XRCC1* Arg399Gln and XRCC3* Thr399Met genes.

		CAs±SD (%)	CTA±SD(%)	CSA±SD(%)
XRCC1	*Arg/Arg*	1.33±0.76	0.71±0.69	0.63±0.71
	Arg/Gln	1.8±1.089	1.00±0.65	0.80±1.06
	Gln/Gln	2.14±0.38*	1.43±0.53	0.71±0.76
XRCC3	*Thr/Thr*	1.50±0.91	0.64±0.66	0.91±0.07
	Thr/Met	1.88±0.84	0.94±0.74	0.94±1.01
	Met/Met	1.83±0.75	1.16±0.40	0.83±0.75

CA - chromosomal aberrations (total), CTA - chromatid-type aberrations, CSA - chromosome-type aberrations,
*(p<0.05) ** (p<0.01), SD - standard deviation

were significantly lower than in those with heterozygous Arg/Gln and variant *Gln/Gln* genotypes. It could indicate the possible role of *XRCC1* in DNA repair caused by chromium.

The tendency for increasing CAs frequencies in association with the variant Gln allele in *XRCC1* Arg-399Gln is in accordance with Skjelbred *et al*. (2006) and Mateuca *et al*. (2008) and is quite logical, as the variant Gln allele is associated with significantly decreased base excision repair capacity in healthy donors. Grlickova-Duzevik *et al*. (2006) investigated the role of *XRCC1* gene for structural chromosomal aberrations on CHO cells treated by lead and chromate. They found out that the total amount of Cr(VI)-induced chromosome damage was exacerbated by *XRCC1* deficiency, chromatid and isochromatid lesions being the most prominent aberrations. In addition, *XRCC1* deficiency resulted in a dramatic increase in the number of chromatid exchanges, indicating that *XRCC1* is involved in protection from lead chromate–induced chromosome instability. Recently, Musak *et al*. (2006) detected higher level of chromosomal aberrations in association with variant Gln allele in *XRCC1* Arg399Gln, as assayed for in cytostatic-exposed medical staff.

In conclusion, our study does not show any dramatic influence of polymorphisms in *XRCC3* gene on chromosomal aberrations in welders following chromium exposure. We report here a clear association between genetic polymorphism of *XRCC1* gene and chromosomal damage and this finding is in agreement with similar population studies and comply well with functional study.

The goal of most studies dealing with polymorphism of DNA repair genes is looking for the tolls for monitoring of individual susceptibility to mutagens and carcinogens in order to protect susceptible individuals. Our study representing only one portion of the puzzle of knowledge needed for developing of optimal and effective monitoring of environmental and occupational exposure in respect to individual characteristics.

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