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Modulation of DNA repair capacity and mRNA expression levels of XRCC1, hOGG1 and XPC genes in styrene-exposed workers

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ABSTRACT

Decreased levels of single-strand breaks in DNA (SSBs), reflecting DNA damage, have previously been observed with increased styrene exposure in contrast to a dose-dependent increase in the base-excision repair capacity. To clarify further the above aspects, we have investigated the associations between SSBs, micronuclei, DNA repair capacity and mRNA expression in XRCC1, hOGG1 and XPC genes on 71 styrene-exposed and 51 control individuals. Styrene concentrations at workplace and in blood characterized occupational exposure. The workers were divided into low (below 50 mg/m^3) and high (above 50 mg/m^3) styrene exposure groups. DNA damage and DNA repair capacity were analyzed in peripheral blood lymphocytes by Comet assay. The mRNA expression levels were determined by qPCR. A significant negative correlation was observed between SSBs and styrene concentration at workplace ($R=-0.38$, $p=0.001$); SSBs were also significantly higher in men ($p=0.001$). The capacity to repair irradiation-induced DNA damage was the highest in the low exposure group (1.34 ± 1.00 SSB/10^9 Da), followed by high exposure group (0.72 ± 0.81 SSB/10^9 Da) and controls (0.65 ± 0.82 SSB/10^9 Da). The mRNA expression levels of *XRCC1*, *hOGG1* and *XPC* negatively correlated with styrene concentrations in blood and at workplace ($p<0.001$) and positively with SSBs ($p<0.001$). Micronuclei were not affected by styrene exposure, but were higher in older persons and in women ($p<0.001$). In this study, we did not confirm previous findings on an increased DNA repair response to styrene-induced genotoxicity. However, negative correlations of SSBs and mRNA expression levels of *XRCC1*, *hOGG1* and *XPC* with styrene exposure warrant further highly-targeted study.

Introduction

Styrene is a monomer extensively used in chemical industries for the production of various plastics and polyester resins and also represents an environmental contaminant (IARC, 2002). Styrene and its primary reactive metabolite styrene-7,8-oxide (SO), the substrate suggested to impart main genotoxic effect, have been classified as possible (2B) and probable (2A) human carcinogens (IARC, 1994). Styrene reportedly induces a wide spectrum of DNA adducts (Vodicka et al., 2002) that may be repaired via different repair pathways, with assumption of base-excision repair (BER) being the major pathway. However, the involvement of nucleotide excision repair and the overlapping role of repair enzymes does not exclude the role of XP proteins in the repair of DNA damage by styrene or its metabolites (Shimizu et al., 2003; Dusinska et al., 2006).

Abbreviations: SO, Styrene-7,8-oxide; BER, Base-excision repair; PBL, Peripheral blood lymphocytes; Da, Daltons; γ-irradiation, Gamma irradiation; EndoIII sites, SSBs endonuclease III sites; MN, Micronuclei; XRCC1, X-ray repair cross-complementing protein 1; hOGG1, 8-hydroxyguanine DNA glycosylase; XPC, Xeroderma pigmentosum, complementation group C; SSB, Single-strand breaks; bp, Base pair; B2M, Beta-2-Microglobulin; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; PPIA, Peptidyl-prolyl isomerase A (cyclophilin A); qPCR, Quantitative PCR; SD, Standard deviation.

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Several studies have indicated that individual susceptibility factors, including DNA repair capacity, metabolism and variants in the genes involved may modulate the genotoxicity of xenobiotics (Norppa, 2003, 2004; Vodicka et al., 2004a,c, 2006a). Interestingly, decreased DNA damage, measured as single-strand breaks (SSBs), has been observed with increased styrene exposure in contrast to the BER rates including removal of oxidative-damage, which has been shown to increase in a concentration-dependent manner (Vodicka et al., 2004c).

In order to further elucidate the DNA damage and repair caused by styrene exposure, we have chosen a new population occupationally exposed to styrene at workplace and matched unexposed controls. In this population, we measured SSBs as a marker of DNA damage, frequency of micronuclei (MN) as an indicator of chromosomal damage and studied DNA repair capacity and, to further understand the phenomenon we also measured mRNA level of three repair genes, *XRCC1*, *hOGG1* and *XPC*. Genetic variants in these genes have previously been shown to modulate BER rates in humans (Vodicka et al., 2007).

Materials and methods

Subjects

The styrene-exposed group consisted of 71 workers employed in hand lamination; the mean length of occupational exposure was 5.2 ± 4.0 years (mean ± SD). Fifty-one workers employed as mechanics in a local car plant represented the control group. Thirteen individuals out of 122 (i.e. 10.7%) were also included in the previous sampling (Vodicka et al., 2004c). The set of analyses undertaken was not feasible or successful for all investigated individuals, thus the actual number of observations is shown for each particular parameter in the respective Table and/or Figure. The differences in the styrene exposure were reflected by the stratification of the studied group into three sub-groups according to the level of styrene concentration at workplace. In the control group, the styrene concentration was below the limit of detection. The exposed group was arbitrarily divided into those with low styrene exposure (below 50 mg/m^3) and with the high styrene exposure (above 50 mg/m^3; Table 1). The design of the study was approved by the local ethical committee of Public Health Institute in Usti nad Orlici, Czech Republic. The sampling of biological material was carried out according to the Helsinki declaration.

Styrene exposure at workplace and in blood

The concentration of airborne styrene at workplace was determined by personal dosimeters at the day of sampling (Vodicka et al., 1995). Styrene in blood was determined as previously described (Vodicka et al., 1995, 2001b).

SSBs in DNA

The levels of DNA damage were measured in peripheral blood lymphocytes (PBL) of both the exposed and the control individuals by means of the alkaline version of the comet assay. Using this assay alkali-labile sites may represent alkali-labile DNA adducts, oxidized bases, abasic sites, true DNA breaks as well as transient gaps appearing in the DNA during DNA repair (Vodicka et al., 2006b). The blood samples were kept on ice until processed. PBL were separated using Ficol gradient from the whole blood, rewashed with PBS, re-suspended in low melting point agarose and layered on microscope slides, followed by lysis for 1 h at 4 °C (lysis solution: 2.5 M NaCl, 100 mM EDTA and 10 mM Tris, with 1% Triton X-100, pH 10). In the next step, all slides were treated with alkaline buffer (300 mM NaOH, 1 mM EDTA, pH 13) for 40 min. The electrophoresis was carried out at 25 V, 300 mA for 30 min at 4 °C. All slides were then washed twice with neutralizing buffer (0.4 M Tris, pH 7.5).

To determine abasic sites and oxopyrimidines more specifically, we measured on parallel slides endonuclease III-sensitive sites by incubating lysed nucleoids with endonuclease III enzyme for 45 min at 37 °C. After a 40 min unwinding period, electrophoresis was carried out as above.

For the scoring, slides were stained with ethidium bromide (0.01 ng/μl, 20 μl per agar) and evaluated by an image-analysis system using the comet module of Lucia G image software (Laboratory Imaging, Czech Republic). Fifty randomly selected nuclei per slide were analyzed and the tail DNA percentage was used for calculating amount of DNA breaks according to the calibration of the method with X-ray irradiation and expressed as SSBs/10^9 Da (Collins et al., 1996, 2001; Vodicka et al., 2001a).

DNA repair rates

DNA repair capacity for removal of γ-irradiation-induced SSBs (i.e. BER rates) has been described in details elsewhere. Briefly, PBL,

Table 1
Characteristics of the study population and characterization of styrene exposure. Exposure biomarkers are reported as mean values ± SD, range and median.

		All subjects	Controls	Low exposed	High exposed	p-value[a]
Age	N	122	51	28	43	ns
	Mean ± SD	39 ± 12	40 ± 12	41 ± 12	37 ± 11	
	Range	21–64	26–62	26–64	21–60	
	Median	36.5	37	37	34	
Sex	N males	95	41	28	26	<0.001
	N females	27	10	0	17	
Smoking status	N smokers	72	35	14	23	ns
	N non-smokers	50	16	14	20	
Exposure (Styrene in air; mg/m^3)	N	122	51	28	43	<0.001
	Mean ± SD	50.3 ± 70.3	0.0 ± 0.0	4.9 ± 5.8	139.4 ± 40.2	
	Range	0–238.0	0.0–0.0	0.0–18.0	61.0–238.0	
	Median	0.0	0.0	0.0	152.0	
Years of exposure	N	122	51	28	43	<0.001
	Mean ± SD	3.2 ± 4.0	0.5 ± 1.9	6.9 ± 4.0	4.0 ± 3.6	
	Range	0.0–14.0	0.0–12.0	1.0–13.0	0.5–14.0	
	Median	1.5	0.0	5.3	2.0	
Styrene in blood (mg/l)	N	84	15	27	42	<0.001
	Mean ± SD	1.03 ± 0.98	0.27 ± 0.25	0.41 ± 0.45	1.71 ± 0.94	
	Range	0.00[b]–3.94	0.00[b]–0.76	0.00[b]–2.29	0.33–3.94	
	Median	0.60	0.26	0.31	1.53	

N — number of individuals, SD — standard deviation, ns — not significant.
[a] Level of significance (K–W test).
[b] Value 0 for styrene concentration in blood represents the values below the detection limit, i.e. 0.1 mg/l.