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Biomarkers of nucleic acid oxidation, polymorphism in, and expression of, *hOGG1* gene in styrene-exposed workers

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ABSTRACT

This study investigated nucleic acid oxidation associated with styrene exposure, mRNA expression levels of *hOGG1* gene and the role of the genetic polymorphism *Ser326Cys* of human 8-oxoguanine DNA N-glycosylase 1 (*hOGG1*) in 60 styrene-exposed workers and 50 unexposed clerks. Biomarkers of exposure (styrene in blood, mandelic and phenylglyoxylic acids and 4-vinylphenol in urine) and urinary biomarkers of nucleic acid oxidation, namely 8-oxo-7,8-dihydro-2′-deoxyguanosine (U-8-oxodGuo), 8-oxo-7,8-dihydroguanosine (U-8-oxoGuo) and 8-oxo-7,8-dihydroguanine (U-8-oxoGua) were determined by liquid chromatography–tandem mass spectrometry. The levels of 8-oxodGuo adduct and 2′-deoxyguanosine (dGuo) were measured by HPLC in DNA from white blood cells (WBC). Genomic DNA and RNA from blood samples were used to characterize the *Ser326Cys* polymorphism and the mRNA expression levels of the *hOGG1* gene, respectively, by PCR-based methods. Exposed workers showed lower values of 8-oxodGuo/10^5 dGuo ratio in WBC-DNA but higher concentrations of U-8-oxoGuo compared to controls ($p = 0.002$ and $p = 0.008$, respectively, *t*-test for independent samples). In the whole group, all urinary biomarkers of nucleic acid oxidation correlated with both the sum of mandelic and phenylglyoxylic acids (*rho* > 0.33, $p < 0.0001$) and 4-vinylphenol (*rho* > 0.29, $p < 0.001$), whereas 8-oxodGuo/10^5 dGuo in WBC showed a negative correlation with exposure parameters (*rho* < −0.24, $p < 0.02$). Subjects bearing the *hOGG1 Ser/Ser* genotype showed lower values of 8-oxodGuo/10^5 dGuo in WBC than those with at least one variant *Cys* allele (0.34 ± 0.16 *vs* 0.45 ± 0.21, $p = 0.008$). In the subgroup of *hOGG1 Ser/Ser* subjects, laminators showed lower levels of WBC 8-oxodGuo/10^5 dGuo ratio and significantly higher concentrations of U-8-oxoGua than controls ($p = 0.07$ and $p = 0.01$, respectively, *t*-test for independent samples). Interestingly, workers showed higher levels of *hOGG1* expression compared to controls ($p < 0.0005$). Styrene exposure seems to be associated with oxidation damage to nucleic acids, particularly to RNA and with an induction of the BER system.

1. Introduction

Styrene is a monomer widely used in the production of polymers, plastics and synthetic rubber. In humans, it is converted to styrene-(7,8)-oxide (7,8-SO) via the cytochrome P450 monooxygenase system (Nakajima et al., 1994). It is generally thought that most of styrene-induced genotoxicity is due to its electrophilic metabolite 7,8-SO, a highly reactive epoxide. Although styrene and 7,8-SO are known to induce both DNA adducts and DNA strand breaks in exposed workers, a recent re-evaluation of various genotoxic endpoints highlighted several inconsistencies in the overall current knowledge (Henderson and Speit, 2005; Vodicka et al., 2006). In

Abbreviations: hOGG1, human 8-oxoguanine DNA N-glycosylase 1; 8-oxodGuo, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; 8-oxoGua, 8-oxo-7,8-dihydroguanine; dGuo, 2′-deoxyguanosine; WBC, white blood cells; MTH1, mutT homolog-1; BER, base excision repair; 7,8-SO, styrene-(7,8)-oxide; MA, mandelic acid; PGA, phenylglyoxylic acid; 4-VP, 4-vinylphenol; LC–MS–MS, liquid chromatography–tandem mass spectrometry.

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addition, the outcomes from cancer epidemiological studies were also inconclusive (Kogevinas et al., 1993). However, the International Agency for Research on Cancer (IARC) classified styrene as possibly carcinogenic with limited evidence for carcinogenicity in humans and experimental animals, whereas 7,8-SO as probably carcinogenic to humans (IARC, 1994).

A hypothesis has been postulated that oxidative stress arising as an imbalance between oxidant and antioxidant molecules may also contribute to the genotoxic effects of styrene (Marczynski et al., 2000). *In vitro* studies have demonstrated that exposure to styrene or 7,8-SO induces increased lipid peroxidation and DNA oxidation as well as glutathione depletion (Chakrabarti et al., 1993; Vettori et al., 2005). The guanine moiety of nucleotides represents one of the main targets for hydroxyl radicals and, depending on the molecular context (2′-deoxyribonucleotides, ribonucleotides, DNA, RNA), oxidized guanine may undergo different repair pathways resulting in different extracellular reaction products (Lunec et al., 2002; Cooke et al., 2008). 8-Oxo-7,8-dihydroguanine (8-oxoGua) in DNA is selectively cleaved by specific glycosylases of the base excision repair (BER) system, including the polymorphic 8-oxoguanine DNA N-glycosylase 1 (hOGG1) (Cooke et al., 2003). Alternatively, oxidized guanine may be released from DNA as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) by an endonuclease-nucleotidase-based DNA repair system (Bessho et al., 1993). The same molecule is generated by the enzyme MTH1 (Tsuzuki et al., 2001) as product of repair of oxidized 2′-deoxyguanosine triphosphate in the cellular 2′-deoxyribonucleotide pool and by the nucleotide excision repair (NER) system, which releases oligonucleotides containing 8-oxodGuo (Patel et al., 2007). 8-Oxo-7,8-dihydroguanosine (8-oxoGuo) may originate from oxidized guanine in RNA, probably as a result of the turnover of the molecule, rather than as product of RNA repair mechanisms, that have not yet been well characterized (Nunomura et al., 2006). The turnover or repair of RNA may be responsible also for the generation of extracellular 8-oxoGua (Evans and Cooke, 2004).

In humans, 8-oxodGuo has been extensively studied either in DNA isolated from white blood cells (WBC) or as free urinary deoxynucleoside, although some inconclusive results have been reported in the field of occupational end environmental exposures (Pilger and Rudiger, 2006). More recently, the advent of liquid chromatography–tandem mass spectrometry (LC–MS–MS) has enabled the determination of oxidatively modified guanine derivatives in urine samples (U-), such as U-8-oxodGuo, U-8-oxoGuo, and U-8-oxoGua (Weimann et al., 2002). Such an approach limits the risk of artifactual oxidation during pre-analytical phases, owing to the minimal sample manipulation prior to injection. The combined evaluation of oxidized guanine derivatives both in DNA from white blood cells (WBC-DNA) and in urine may allow a better understanding of genotoxic mechanisms at the molecular level. DNA oxidation may be modulated by germ line variants in DNA repair genes, the most prominent being hOGG1. For this enzyme, a functional genetic polymorphism is known (*Ser326Cys*) and the variant allele distributes in Caucasian with a prevalence of about 20% (Marchand et al., 2002). Although the association between *hOGG1* genotype and the enzyme activity of OGG1 has not been definitely proven so far (Weiss et al., 2005), experimental investigations demonstrate that the hOGG1 Cys326 isozyme has impaired function (by about 2-fold) compared to the Ser326 isoform (Luna et al., 2005; Bravard et al., 2009). In agreement with these data, a recent epidemiological study (Vodicka et al., 2007) has shown that subjects with the *hOGG1 Cys/Cys* genotype exhibit a 50% lower DNA repair capacity when compared to *hOGG1 Ser/Ser* subjects.

The present study was carried out to investigate the levels of oxidized guanine derivatives in a group of styrene-exposed workers with accurately characterized internal dose levels, and an unexposed control group. As an additional aim, we evaluated the modulating role of both gene expression and genetic polymorphism of the *hOGG1* gene on oxidatively generated DNA damage associated with styrene exposure. In particular, we evaluated the relationships between the levels of the oxidized guanine in WBC-DNA (determined as 8-oxodGuo/10^5 dGuo) that is the relevant substrate for hOGG1 activity and the urinary concentrations of the reaction product 8-oxoGua in subjects classified by the *hOGG1 Ser326Cys* polymorphism.

Table 1
Characteristics of the studied population and characterization of styrene exposure. Exposure biomarkers are reported as median and range.

	Controls ($n = 50$)	Exposed ($n = 60$)
Sex (male/female)	39/11	42/18
No. of current/never-smokers	13/37	26/34
Age (years)	40.0 ± 12.1 (26–62)	37.6 ± 11.1 (21–64)
Years of employment	–	4.0 ± 3.4 (1–14)
Styrene air, mg/m^3	n.d.	107.4 ± 66.7
Blood styrene, mg/L	0.20 (n.d. to 0.43)	1.20 (n.d. to 3.94)
MA + PGA, mg/g creatinine	0.47 (0.17–3.24)	286.7 (4.16–2022)
4-VP, mg/g creatinine	0.19 (0.01–4.08)	3.39 (0.19–22.6)

Note: 1 ppm of styrene is equal to 4.25 mg/m^3. MA + PGA: mandelic acid + phenylglyoxylic acid; 4-VP: 4-vinylphenol; n.d.: not detectable.

2. Materials and methods

2.1. Subjects and sampling

Sixty styrene-exposed workers employed in two plastics lamination plants in the same geographical area and 50 unexposed clerks volunteered to participate in the study. Confounding factors, like X-rays, medical drug treatment, dietary and lifestyle were carefully controlled by detailed questionnaire. The study was conducted on healthy individuals and exclusion criteria comprised a recent exposure to X-rays, current drug use or viral infections experienced in the last 3 months. The main characteristics of the studied population are reported in Table 1. The local Ethical Committee approved the study protocol and the participating subjects provided their written informed consent. The sampling of biological material was carried out according to the Helsinki Declaration (WHO, 1964).

Spot urine samples (50 mL) were collected at the end of the shift, divided into two aliquots and frozen at −20 °C until analysis. Blood samples (40 mL) were collected from all subjects in the middle of the work shift (ensuring saturation) on the same day as collection of urine samples.

2.2. Chemicals

Styrene (purity 99%), DL-mandelic acid (MA, 98%), phenylglyoxylic acid (PGA, 98%), 2′-deoxyguanosine (dGuo, 99–100%), and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo, 98%) were purchased by Sigma–Aldrich (Taufkirchen, Germany and Milan, Italy). 8-Oxo-7,8-dihydroguanosine (8-oxoGuo, 98%) and 8-oxo-7,8-dihydroguanine (8-oxoGua, 90%) were from Cayman (MI, USA). Isotopically labeled compounds used as either internal standards (ISs), *i.e.* [$^{13}C_1$, $^{15}N_2$]8-oxoGua ($^{13}C_1$ 98%, $^{15}N_2$ 98%) and [$^{15}N_5$]Guo (96–98%, used as IS for 8-oxoGuo), or for the synthesis of ISs, *i.e.* [$^{15}N_5$]dGuo (96–98%), were obtained from Cambridge Isotope Laboratories Inc. (MA, USA). [$^{15}N_5$]8-oxodGuo had been synthesized from [$^{15}N_5$]dGuo according to Hu et al. (2004), with minor modifications. All standards were used without further purification.

2.3. Styrene exposure at the workplace

The concentration of airborne styrene at the workplace was determined by personal dosimeters on the day of the sampling, as previously described (Vodicka et al., 1995). Results are summarized in Table 1.

2.4. Exposure biomarkers

Styrene in the blood was determined as previously described (Vodicka et al., 1995, 2001). Styrene metabolites, namely MA, PGA, and 4-VP were determined by LC–MS–MS as previously described (Manini et al., 2002). Concentrations of urinary metabolites were expressed as a function of creatinine concentration (mg/g creat.), measured by the method of Jaffe (Kroll et al., 1986). Sample with creatinine concentrations lower than 0.3 g/L or higher than 3.0 g/L were excluded from statistical analysis according to the American Conference of Governmental Industrial Hygienists recommendation (ACGIH, 2004). For quantitative analyses, calibrations were performed in a matrix by spiking pooled urine samples from non-exposed subjects with appropriate standard mixtures. The concentrations of styrene in blood and