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Influence of genetic polymorphisms of styrene-metabolizing enzymes on the levels of urinary biomarkers of styrene exposure

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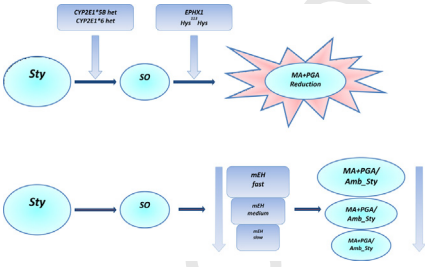
HIGHLIGHTS

- CYP2E1*5B/*6 heterozygotes excrete less urinary styrene metabolites than wild type.
- Subjects carrying the EPHX1 (codon 113) exhibit reduced urinary [MA + PGA].
- Urinary [MA + PGA] is modulated by predicted epoxide hydrolase activity.
- Styrene individual metabolic variability could lead to underestimation of exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Styrene exposure is still present in different occupational settings including manufacture of synthetic rubber, resins, polyesters and plastic. The aim of this work was to investigate the effects of polymorphic genes CYP2E1, EPHX1, GSTT1, and GSTM1 on the urinary concentrations of the styrene metabolites mandelic acid (MA), phenylglyoxylic acid (PGA) and on the concentration ratios between (MA + PGA) and urinary styrene (U-Sty) and airborne styrene (A-Sty), in 30 workers from two fiberglass-reinforced plastic manufacturing plants and 26 unexposed controls. Personal air sampling and biological monitoring results revealed that sometimes exposure levels exceeded both the threshold limit value (TLV) and the biological exposure index (BEI) suggested by the American Conference of Governmental Industrial Hygienists. A significantly reduced excretion of styrene metabolites (MA + PGA) in individuals carrying the CYP2E1*5B and CYP2E1*6 heterozygote alleles, with respect to the homozygote wild type, was observed only in the exposed group. A reduction was also detected, in the same group, in subjects carrying the slow allele EPHX1 (codon 113), through the lowering of (MA + PGA)/urinary styrene concentration ratio. In addition, the ratio between MA + PGA and the personal airborne styrene concentration appeared to be modulated by the predicted mEH activity, in the exposed group, as evidenced by univariate linear regression analysis. Our results confirm some previous hypotheses about the role of the polymorphism of genes coding for enzymes involved in the styrene detoxification pathway: this may significantly reduce the levels of excreted metabolites and therefore it must be taken into account in the interpretation of the biological monitoring results for occupational exposure.

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1. Introduction

Styrene is an organic compound used in the fabrication of reinforced-polyester plastic composites. Human exposure occurs in many industrial settings like hand-lamination plants, production of glass-reinforced plastic products and boat building (Miller et al., 1994).

Due to improved industrial hygiene and more stringent regulations, occupational exposure to styrene has steadily declined, but the highest exposures have been measured in the reinforced plastic industry (Rueff et al., 2009). In this sector, exposure occurs mainly through the inhalation of its vapors during the lamination and curing steps (Miller et al., 2001).

In humans styrene detoxification pathway is started up by cytochrome P-450-(CYP)-mediated oxidation into a high reactive and electrophilic molecule, 7,8-styrene oxide (SO) which is considered to be directly responsible for the genotoxic effect. SO is then hydrolyzed by microsomal epoxide hydrolase (mEH) in phenyl ethylene glycol which is further metabolized to mandelic acid (MA) and phenylglyoxylic acid (PGA) (Rihs et al., 2008). About 90% of the styrene uptake is metabolized and excreted in the urine as MA and PGA (Vodicka et al., 2001). In humans there is also a minor route, accounting for less than 1% of the amount inhaled, catalyzed by glutathione S-transferases (GSTs) family enzymes, where SO is conjugated with reduced glutathione. These conjugates are then acetylated and excreted in the urine as specific phenyl hydroxyethyl mercapturic acids (PHEMAs) (Summer and Fennel, 1994). A small fraction of styrene escapes metabolic transformation and is eliminated unchanged through the urine (Prieto et al., 2002). Measurement of the urinary metabolites MA and PGA at the end of the work shift is the most commonly used method for the biological monitoring of exposure, for which the American Conference of Governmental Industrial Hygienists (ACGIH) adopted a biological exposure limit (BEI) for the sum of the concentrations of the two metabolites, measured at the end of work shift, of 400 mg/g of creatinine (ACGIH, 2014). Several in vitro and in vivo studies have reported that styrene and SO were both able to induce DNA adducts and DNA strand break in exposed workers (Costa et al., 2012; Teixeira et al., 2010; Vodicka et al., 2006); moreover the International Agency for the Research on Cancer (IARC) classified these substances respectively as possible (group 2B) and probably (group 2A) carcinogens to humans (IARC, 2002). Numerous enzyme systems are involved in the styrene metabolism: several studies showed that genetically determined individual variations of these enzymes, through single-nucleotide polymorphisms (SNPs), may affect their activity influencing the detoxification pathway (Vodicka et al., 2001). In particular a reduced expression of mRNA, as well as a decreased excretion of the styrene metabolites, was found in individuals carrying the CYP2E1*5B heterozygote variant respect the wild type homozygote. A similar tendency was observed for the variant CYP2E1*6, but without statistical significance (Prieto-Castelló et al., 2010). Moreover, Haufroid et al. (2002) have reported a lower excretion of the urinary metabolites MA, PGA, and PHEMAs in subjects carrying the heterozygote allele CYP2E1*6 associated a reduced CYP2E1 inducibility. Other authors also studied the impact of EPHX1 polymorphisms on styrene metabolism. For this gene two polymorphic sites have been observed in exons 3 ($Tyr^{113}His$) and 4 ($His^{139}Arg$). Based on in vitro studies this variant allele were associated to a reduced (−39%) and increased activity (+25%) respectively (Hassett et al., 1994). A significant correlation between the low activity profile of EPHX1, combined with the CYP2D6 genotype, and the urinary metabolites MA + PGA was found above 50 ppm of styrene exposure (Ma et al., 2005). Some studies have reported the involvement of GSTs polymorphisms in the variation of PHEMAs excretion in urine (De Palma et al., 2001; Haufroid et al., 2002) but few data are available about the effect of the GSTs polymorphism on the MA + PGA excretion in exposed workers. Teixeira et al. (2004) reported a possible modulating effects of GSTM1 genotype on the MA + PGA excretion level, in a group of 28 workers employed in two small reinforced plastic plants, with levels of metabolites twice higher in GSTM1 null individuals after exposure to low levels. About the effect of GSTT1 genetic polymorphism on styrene metabolism, De Palma et al. (2001) have reported an increased excretion level of MA + PGA in GSTT1 positive genotypes compared to the negative ones. The aim of this work was to evaluate the influence of individual genetic polymorphisms on styrene-metabolizing enzymes activity, observing the effects of a single or multiple allelic variants on the level of urinary metabolites following occupational exposure, to styrene, but independently from the exposure levels.

2. Material and methods

2.1. Subjects and study design

For this study 30 styrene exposed workers employed in two different manufacturing sites of central Italy and 26 controls were selected. A questionnaire was administered to workers and control subjects to be enrolled in order to collect information on their life habits and use of drugs, especially to gather information on possible source of exposure to styrene outside the workplace. Blood samples for DNA analysis were taken during the routine medical surveillance.

2.2. Personal air monitoring

2.2.1. Chemicals and supplies

Radiello® (ALT CTRL R) passive air samplers were supplied by Supelco (Bellefonte, PA, USA). All reagents were of high purity analytical grade. The analytical reference standard of styrene was purchased from Riedel-de Haën (Buchs, Switzerland), deuterium labelled styrene (d8) from Isotec, Inc. (Miamisburg, OH, USA). Carbon disulfide was purchased from Sigma–Aldrich (Steinheim, Germany). A Milli-Q water purification system (Milli-pore, Bedford, MA, USA) was used to supply high purity de-ionized water. A polyethylene glycol capillary column DB-WAXetr 123–7334 (30 m × 0.32 mm i.d., 1.00 μm film thickness; J&W California, USA) was used for the chromatographic separation. Pure Helium (purity level 99.999%) was used as GC carrier gas (Air Liquid, Milan, Italy).

2.2.2. Instrumental analysis

Quantitative determinations of airborne styrene were performed by a gas chromatograph (6890N Agilent Technologies) coupled with a single quadrupole mass spectrometer (5973 MSD System, Agilent Technologies). The peak areas were integrated by the ChemStation Software (Agilent Technologies). The injection temperature (split ratio 1:10) was set at 230 °C. Helium carrier gas flow rate in the analytical column was 1.5 mL/min. The column oven temperature was initially set at 50 °C, then raised to 120 °C with 2.5 °C/min increments. Electron impact spectra were obtained with electron energy of 70 eV. Detection and source temperature were set at 150 °C and 230 °C, respectively. Detection was performed in the single-ion monitoring (SIM) mode. The mass-to-charge ratios (m/z) selected were 104 for styrene and 112 for styrene (d8), used as internal standard. Working calibration standard solutions, blanks and samples were analyzed in triplicate and the average was used. The detection limit (LOD) and the lower limit of quantification (LOQ) were 0.7 $\mu g/m^3$ and 1.9 $\mu g/m^3$ respectively. The intra- and inter-assay precision of the method was below 10%.

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