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Influence of MRP1 G1666A and GSTP1 Ile105Val genetic variants on the urinary and blood arsenic levels of Turkish smelter workers

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ABSTRACT

To understand the cellular mechanisms responsible for arsenic metabolism and transport pathways plays a fundamental role in order to prevent the arsenic-induced toxicity. The effect of MRP1 G1666A and GSTP1 Ile105Val polymorphisms on blood and urinary arsenic levels were determined in 95 Turkish smelter workers. Blood and urinary arsenic concentrations were measured by GF-AAS with Zeeman correction and gene polymorphisms were investigated by PCR-RFLP method. The mean blood and urinary arsenic levels were 21.60 ± 12.28 μg/L and 5.58 ± 4.37 μg/L, respectively. A significant association between MRP1 1666A allele and urinary arsenic levels was found ($p = 0.001$). GSTP1 Ile105Val polymorphism was detected not to be associated with either blood or urinary arsenic levels ($p = 0.384$, $p = 0.440$, respectively). Significant association was also detected between MRP1A$^-$/GSTP1Val$^-$ genotypes and urinary arsenic levels ($p = 0.001$). This study suggested that MRP1 G1666A alone and, also, combined with GSTP1 Ile105Val were associated with inter-individual variations in urinary arsenic levels, but not with blood arsenic levels.

1. Introduction

Metalloid element arsenic is a widespread environmental pollutant present in the air, water and soil due to the natural and anthropogenic activities (Rosen and Liu, 2009). It enters the human body mainly via drinking water. Consumption of foods such as rice cultivated with arsenic contaminated ground water and/or occupational exposures are the other sources of exposure (Bhattacharjee et al., 2013; Cascio et al., 2011). Chronic exposure to arsenic is associated with neurological disorders, peripheral vascular disease and diabetes mellitus as well as cancers of skin, bladder, liver, lung and kidney (Rosen and Liu, 2009). The International Agency for Research on Cancer (IARC) classified arsenic as group 1 carcinogen. Arsenic reveals its toxic effects on various organs and tissues including skin, lungs, liver and blood systems through several mechanisms (Bhattacharjee et al., 2013). The most significant of which is the reversible combination with sulfhydryl groups due to its high affinity to sulfhydryl compounds. Although it can be found four oxidation states (−3, 0, +3, +5) in the environment, the pentavalent arsenate (AsV) and trivalent arsenite (AsIII) are the biologically important oxidation forms of arsenic (Faita et al., 2013).

Inorganic arsenic is mainly biomethylated to various metabolites by a sequence of two distinct enzymatically catalyzed reactions in human liver, and excreted into urine and feces. Because only arsenite can be methylated, arsenate is first reduced to the arsenite enzymatically by human liver (Watanabe and Hirano, 2013). In the literature, there are two distinct arsenic methylation pathways: oxidative methylation and reductive methylation. Both in oxidative and reductive methylation pathways, arsenic (+3 oxidation state) methyltransferase (AS3MT) catalyzes the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to trivalent arsenicals (Bhattacharjee et al., 2013). Methylation has been considered as a detoxification process since it increases the clearance of arsenic from body (Leslie, 2012). The ratios of inorganic arsenic and its methylated metabolites in the urine are 10–30% inorganic arsenic, 10–20% monomethylated metabolites and 60–80% dimethylated metabolites (Rossman, 2003). If the levels of urinary total dimethylated metabolites are low compared to total monomethylated metabolites, overall excretion rates of arsenic would be low and arsenic retention in tissues would be increased (Leslie, 2012).

To understand and prevent the arsenic-induced toxicity, studies have been conducted on the cellular mechanisms responsible for arsenic transport pathways since arsenic toxicity occurs due to its accumulation ratios and the intracellular arsenic accumulation is associated with inorganic arsenic uptake and efflux. Regarding efflux, multidrug resistance protein 1 (MDR1; also known as

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P-glycoprotein, P-gp) and multidrug resistance-associated proteins 1 and 2 (MRP1/2) pump out arsenic of the cell (Yoshino et al., 2011). There have been studies related to the human MRP1 protects tissues from arsenic accumulation and resulting toxicity through the cellular efflux of arsenic–glutathione conjugates (Vernhet et al., 2000). MRP1, also known as ABCC1, is a member of ATP-Binding Cassette (ABC) superfamily (Zhao et al., 2010). It was first cloned in 1992 from the drug-selected human lung cancer cell line H69AR (Cole, 2014). It is a 190 kDa membrane phosphoprotein and is expressed in many drug-resistant tumor cells as well as in normal tissues including lungs, testes and kidney, except the liver (Cole and Deeley, 2006). MRP1 localizes to the basolateral surface of polarized epithelial and endothelial cells (Leslie, 2012) and serves as an efflux carrier (so-called Phase III elimination) of many substrates such as glutathione-, glucuronide-, or sulfate-conjugated molecules produced by Phase II metabolism of many xenobiotics including drugs, antimonial and arsenical oxyanions and other toxicants (Cole and Deeley, 2006).

Zaman et al. (1995) found that MRP1 is a glutathione (GSH)-S-conjugate transporter with the evidences that the extrusion of drug from the cells by MRP1 is prevented by the GSH depletion and, that ATP-dependent transport of GSH-S-conjugates is increased with the overexpression of MRP (Zaman et al., 1995). Vernhet et al. (2001) also observed that buthionine sulfoximine, a potent GSH-depleting agent, caused a decrease in intracellular GSH levels and the accumulation of As_2O_3 was significantly higher in these GSH-depleted GLC4/Sb30 cells due to the impaired efflux of arsenic (Vernhet et al., 2001). Enhanced glutathione S-transferase P1 (GSTP1) levels was found to be significantly correlated with arsenic resistance in Chinese Hamster ovary cells (Müller et al., 1994; Wang and Lee, 1993). Furthermore, in vitro transport studies of MRP1 showed that arsenic triglutathione can be formed by GSTP1 (Deeley et al., 2006). GSH is not only required for the formation of glutathione-conjugates, but also serves as a co-transporter for some chemotherapeutic drugs (Leslie, 2012). GSTs, found in virtually every living species, are important Phase II enzymes involved in the formation of GSH-conjugates, thereby decrease the reactivity of electrophilic substrates of a wide variety of xenobiotics (Gundacker et al., 2010). GSTP1, one of 8 classes of cytosolic GSTs, is expressed in normal and tumor tissues (Vlaykova et al., 2007).

So far, there have been studies in the literature regarding the synergistic role of GSTP1 in the transport of inorganic arsenic as a tri-GSH conjugate by MRP1 (Leslie et al., 2004; Leslie, 2012). The association of GSTP1 Ile105Val gene polymorphism with arsenic metabolism was detected in residents from the arsenic contaminated groundwater areas in Vietnam (Agusa et al., 2012). On the other hand, there has been no study concerning the effect of MRP1 G1666A polymorphism on arsenic metabolism. Also, to the best of our knowledge, the influence of the combination of MRP1 G1666A and GSTP1 Ile105Val polymorphisms on the excretion of arsenic remains unclear. Thus, this is the first study to find out whether MRP1 G1666A alone (rs4148330; GeneID: 4363; Accession Number: NM_004996) and combined with GSTP1 Ile105Val (rs1695; GeneID: 2950; Accession Number: NM_000852) polymorphic characteristics have an effect on blood and urinary arsenic samples.

2. Methods

2.1. Study subjects

The study population comprised 95 Turkish male smelter workers who work in a factory that produces silver from its ores in Kütahya, Türkiye. Workers stating themselves as Turkish were included in the study. Blood and urine samples were provided from workers referred to Ankara Occupational Diseases Hospital for routine control. Written informed consent was obtained from each worker. A small questionnaire for gathering the information including age, exposure time and smoking habit was also given to the individuals. The study design was approved by the institutional ethics committee (approval no: 15-512-12 in 2012). Samplings were performed in accordance with the principles of The Declaration of Helsinki. Blood samples were kept at 4 °C while they were inactive use. Urine samples were kept at −20 °C until analysis.

2.2. DNA extraction and genotyping of MRP1 G1666A and GSTP1 Ile105Val polymorphisms

Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA) anticoagulant whole-blood samples using the QIAamp blood DNA mini-kit (Qiagen, Hilden, Germany) according to the method recommended by the manufacturer. DNA concentration was determined using the PicoGreen dsDNA quantitation kit (Molecular Probes, Eugene, OR) according to the manufacturer's instructions.

Genotyping was carried out using PCR followed by restriction fragment length polymorphism (RFLP). PCR amplification was carried out in a final volume of 50 μL on a Techne Tc 512 PCR System. PCR conditions were as follows: predenaturation for 10 min at 94 °C, followed by 35 cycles of denaturation for 60 s at 94 °C, annealing for 60 s at 60 °C, primer extension for 60 s at 72 °C, and final extension at 72 °C for 5 min.

For detection of the G1666A SNP in the promoter region of the *ABCC1* gene, a 160 bp fragment was amplified by PCR using the following primers: forward: 5′-GCAACAGCATAACTGGCATT-3′ and reverse: 5′-GAGACCTCCCCCCAATCA-3′. Then, 10 microliters of PCR product (160 bp) was digested by *MspI* (New England Biolabs, Hertfordshire, UK) and incubated at 37 °C for overnight. The undigested and digested PCR products were separated by electrophoresis using a 3% agarose gel, stained with ethidium bromide, visualized by an UV trans-illuminator and photographed using a Syngene Monitoring System. Digestion of the PCR product by *MspI* yields fragments that represent the presence of the A allele (160 bp fragment) and the G allele (90 and 70 bp fragments). Digested and undigested PCR products separated using agarose gel electrophoresis were indicated in Fig. 1.

To screen for Ile105Val polymorphism of *GSTP1*, 176 bp fragment containing the Ile105Val polymorphic site was amplified by PCR using the following primers: forward 5′-ACCCCAGGGCTCTATGGGAA-3′ and reverse 5′-TGAGGGCACAAGAAGCCCCT-3′. Then, the PCR product (176 bp) was digested with *BsmAI* restriction enzyme (New England Biolabs, Hertfordshire, UK) and incubated at 55 °C for 4 h. The undigested PCR product and digested products were separated on a 3% agarose gel electrophoresis, visualized by ethidium bromide staining under an ultraviolet illuminator, scanned and photographed using Syngene Monitoring System.

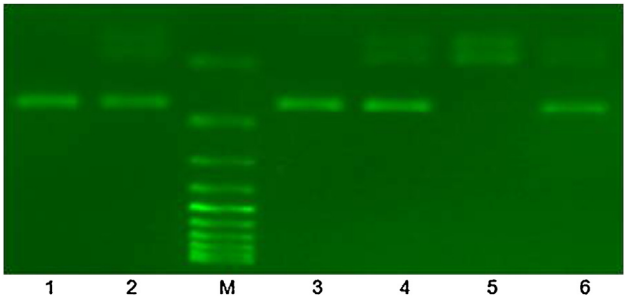

Fig. 1. Representative gel image of digested PCR products with *MspI*. M:100 bp ladder; Lanes 1 and 3: A/A genotype (160 bp); Lanes 2,4 and 6: G/A genotype (160, 90 and 70 bp); Lane 5: G/G genotype (90 and 70 bp).