Food and Chemical Toxicology 90 (2016) 30–35

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Molecular biomarkers of oxidative stress and role of dietary factors in gasoline station attendants

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ARTICLE INFO

Article history:
Received 24 November 2015
Received in revised form
17 January 2016
Accepted 22 January 2016
Available online 29 January 2016

Keywords:
Benzene
Oxidative stress
AGE
AOPP
ROMs
Gasoline station attendants
Mediterranean diet

ABSTRACT

Exposure to benzene promotes oxidative stress through the production of ROS, which can damage biological structures with the formation of new metabolites which can be used as markers of oxidant/antioxidant imbalance.

This study aims to assess modifications in circulating levels of advanced oxidation protein products (AOPP), advanced glycation end-products (AGE) and serum reactive oxygen metabolites (ROMs) in a group of gasoline station attendants exposed to low-dose benzene and to evaluate the influence of antioxidant food intake on these biomarkers of oxidative stress. The diet adopted by the population examined consisted of compounds belonging to the classes of terpenoids, stilbenes and flavonoids, notably resveratrol, lycopene and apigenin.

Ninety one gasoline station attendants occupationally exposed to benzene and 63 unexposed male office workers were recruited for this study. Urinary *trans, trans*-muconic acid (t,t-MA) concentration, determined to assess individual exposure level, resulted significantly higher in exposed workers.

In subjects exposed to benzene, we observed a significant increase ($p < 0.001$) in ROMs and AOPP levels, which were also negatively correlated with fruit and vegetables consumption. By contrast, AGE did not show a significant increase and consequently any relation with antioxidant food intake. Only ROMs, representing a global biomarker of oxidative status, resulted correlated to t,t-MA levels ($p < 0.01$), probably due to low-dose exposure.

Increase of ROS induced by reactive benzene metabolites may promote specific biochemical pathways with a major production of AOPP, which seem to represent a more sensitive biochemical marker of oxidative stress in workers exposed to benzene compared to AGE. Furthermore, this is the first study demonstrating ROMs increment in subject exposed to benzene. These biomarkers may be useful for screening purposes in gasoline station workers and other subjects exposed to low-dose benzene. Moreover, a diet rich in fruits and vegetables demonstrated an inverse association with the levels of oxidative stress markers, suggesting a protective role of antioxidant food intake in workers exposed to oxidant agents.

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; AGE, advanced glycation end-products; ALE, advanced lipoperoxidation end-products; AOPP, advanced oxidation protein products; ARE, antioxidant response element; IARC, International Agency for Research on Cancer; NIOSH, National Institute for Occupational Safety and Health; Nrf2, nuclear factor 2; ROMs, serum reactive oxygen metabolites; ROS, reactive oxygen species; t,t-MA, t,t-muconic acid.

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http://dx.doi.org/10.1016/j.fct.2016.01.017
0278-6915/

1. Introduction

Benzene, an important environmental pollutant widely used in industrial settings, is classified in EU as Carc 1A and as a group 1 human carcinogen by the International Agency for Research on Cancer (IARC).

The American Conference of Governmental Industrial Hygienists (ACGIH) and the National Institute for Occupational Safety and Health (NIOSH) have set an exposure limit value for benzene at 1.6 mg/m^3 (0.5 ppm) and 0.32 mg/m^3 (0.1 ppm) respectively.

Recent studies demonstrated that subjects exposed to benzene air concentrations <0.1 ppm metabolize benzene about nine times more efficiently than heavily exposed workers. This suggests that the health risks associated with low and very low benzene exposures can be considerably greater than those predicted by linear extrapolation from epidemiological studies involving workers exposed to air concentrations of tens to hundreds of ppm (Kim et al., 2006).

The chronic exposure of humans to benzene in work places has been associated with hematotoxicity, leukemia and aplastic anemia, after long-term exposure to high concentrations (Snyder, 2012; Lagorio et al., 2013; Bassig et al., 2015). Moreover, recent studies demonstrated hematological and genotoxic disorders in subjects occupationally exposed to low levels of benzene (Glass et al., 2003; Lan et al., 2004; Smith et al., 2007; Rappaport et al., 2013). However, although it is well established that benzene requires metabolism to induce its effect (McHale et al., 2012), the exact mechanism responsible for its toxicity still remains unclear.

The enzymatic bioactivation of benzene leads to the formation of reactive metabolites, such as phenol, catechol and idroquinone, which play a key role in the production of reactive oxygen species (ROS) (Barreto et al., 2009). Several studies have demonstrated increased ROS levels after benzene exposure *in vivo* and *in vitro* (Ho and Witz, 1997; Winn, 2003). The imbalance between the production of ROS and antioxidants in favor of free radicals causes a state of oxidative stress (El Batsh et al., 2015). Toxic effects are caused through the production of peroxides and free radicals that damage important cellular components - proteins, carbohydrates, lipids and nucleic acids - and may enhance inflammatory response. New compounds and modified structures (which can serve as markers of these mechanisms) are formed, as advanced oxidation protein products (AOPP), advanced glycation end-products (AGE) and advanced lipoperoxidation end-products (ALE) (Kalousova et al., 2005). Most of these products have been extensively investigated in various human tissues and blood and they have been proposed as biomarkers for risk evaluation in workers exposed to benzene (De Palma and Manno, 2014). Risk assessment of residual compounds in food and environmental pollutants has emerged as a significant need for the evaluation of toxicity and hazard index in human populations (Tsakiris et al., 2015).

Furthermore, recently serum reactive oxygen metabolites (ROMs) have been reported to be a reliable biomarker to assess oxidative stress (Chen and Kotani, 2015).

The aim of this study is to assess modifications of AOPP, AGE and ROMs circulating levels, as early markers of oxidative stress, in a group of gasoline station attendants exposed to low dose of benzene.

Additionally, t,t-muconic acid (t,t-MA) in urine, as biomarker of benzene exposure, and the influence of vegetables and fruits consumption, a rich source of exogenous antioxidants, on these biomarkers have also been evaluated.

2. Material and methods

2.1. Study population

A group of 91 men, employed in gasoline stations located in Eastern Sicily, was enrolled for the study and compared with a control group (n = 63) of male office employees with no occupational exposure to benzene. Workers were enrolled in a health surveillance program for the prevention of occupational diseases. Written consent was obtained for participation in this study. A custom-made questionnaire was used to collect information on socio-demographic characteristics (age and Body Mass Index), lifestyle (smoking habit, alcohol consumption, fruits and vegetables intake) and occupational features (lifetime exposure to benzene, use of personal protective equipment) and to exclude known disorders or diseases (job-related diseases, infection or other pathology involving oxidative stress) in the three months preceding the survey.

2.2. Assessment of benzene exposure

Environmental monitoring data, provided by gasoline station managers, indicated benzene levels ≤0.1 ppm as measured at pump site.

Urine samples of both exposed and control group were collected at the end of the work shift, after three consecutive days of exposure, and stored at −80 °C until analysis. Urinary t,t-MA concentration, as a biomarker of benzene exposure, was determined by solid phase extraction followed by high performance liquid chromatography with diode array detection with an Agilent 1200 series HPLC, using a kit produced by Eureka Lab Division (Ancona, Italy); t,t,-MA levels were expressed as μg/ml.

2.3. Evaluation of molecular biomarkers of oxidative stress

AGE and AOPP levels were determined using the methods depicted by Kocak et al. (2009) with some modifications described in a previous study (Costa et al., 2015).

Briefly, for AGE measurement serum samples were diluted 1:50 with phosphate buffered saline (PBS, pH 7.4) and pipetted in a black microtiter plate. Fluorescence intensity with λ_{exc} 350 nm and λ_{em} 440 nm was measured with a Sinergy HT microplate absorbance reader (Biotek, Winooski, USA) and expressed as arbitrary units (AU) per ml.

To measure AOPP serum concentrations, diluted serum samples (200 μl, 1:5 in PBS) were pipetted in a microtiter plate with 10 μl of 1.16M KI and 20 μl acetic acid. Absorbance was measured at 340 nm with a Sinergy HT reader, using a calibration curve with 0–128 μM chloramine T for AOPP quantification.

In order to assess reactive oxygen metabolites, d-ROMS test was used (Diacron International). Absorbance at 505 nm was recorded and measurements were expressed as Carr Units (U CARR) (Hirose et al., 2009).

The coefficient of variation for replicate measurements was <5% for all assays.

2.4. Statistical analysis

Data were analyzed by Prism version 5.01 (GraphPad software, La Jolla, CA, USA) using Student's t test to compare benzene-exposed subjects to control group, while correlation analysis was performed by Spearman test. A $p < 0.05$ was adopted as a limit of significance.