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Low-dose occupational exposure to benzene and signal transduction pathways involved in the regulation of cellular response to oxidative stress

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ABSTRACT

Aims: Benzene metabolism seems to modulate NF-κB, p38-MAPK (mitogen-activated protein kinase) and signal transducer and activator of transcription 3 (STAT3) signalling pathways via the production of reactive oxygen species. This study aims to evaluate the effects of low-dose, long-term exposure on NF-κB, STAT3, p38-MAPK and stress-activated protein kinase/Jun amino-terminal kinase (SAPK/JNK) signal transduction pathways in peripheral blood mononuclear cells in gasoline station attendants. The influence of consumption of vegetables and fruits on these pathways has also been evaluated.
Main methods: A total of 91 men, employed in gasoline stations located in eastern Sicily, were enrolled for this study and compared with a control group of 63 male office workers with no history of exposure to benzene. The exposure was assessed by measuring urinary *trans*,*trans*-muconic acid (t,t-MA) concentration. Quantitative analyses were performed for proteins NF-κB p65, phospho-NF-κB p65, phospho-IκB-α, phospho-SAPK/JNK, phospho-p38 MAPK and phospho-STAT3 using an immunoenzymatic assay.
Key findings: The results of this study indicate significantly higher t,t-MA levels in gasoline station attendants. With regard to NF-κB, phospho-IκB-α and phospho-STAT3 proteins, statistically significant differences were observed in workers exposed to benzene. However, no differences were observed in SAPK/JNK and p38-MAPK activation. These changes were positively correlated with t,t-MA levels, but only phospho-NF-κB p65 was associated with the intake of food rich in antioxidant active principles.
Significance: Chronic exposure to low-dose benzene can modulate signal transduction pathways activated by oxidative stress and involved in cell proliferation and apoptosis. This could represent a possible mechanism of carcinogenic action of chronic benzene exposure.

1. Introduction

Benzene is an industrial and environmental pollutant that is widely used in industrial settings, which is also present in gasoline, engine exhausts, wood and tobacco smoke [30].

Exposure of the general population to benzene by inhalation, representing at least 99% of total personal exposure, is caused by various sources, including vehicle emissions, evaporative loss from gasoline pump stations and cigarette smoke, whereas the secondary route of exposure is its intake with food or water [14,24,42]. Occupational exposure, however, occurs in individuals employed in gasoline production and distribution stations [2]. Others exposed to benzene are traffic policemen and drivers [11].

Benzene is classified as a category 1A carcinogen by the European Union (EU) (Annex VI of Reg. 1272/2008/EC, Dir. 2014/27/EU, Dir. 2004/37/EC) and group 1 human carcinogen by the International Agency for Research on Cancer (IARC). It is widely accepted that benzene can cause haematologic diseases such as leukaemia and myelodysplastic syndrome [17,19,37,39].

With the progressive decrease in both the use of benzene and its environmental and occupational exposure limits, effects of its high concentration at workplace should no longer be a threat. In fact, between 2003 and 2004, the European Agency for Safety and Health at Work

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(EU-OSHA) occupational threshold limit value (TLV) decreased from 3 to 1 ppm (Dir. 2004/37/EC), whereas that of the American Conference of Governmental Industrial Hygienists (ACGIH) decreased from 10 to 0.5 ppm from 1987 to 1999 [1]. At present, the focus has been shifted to the effects of long-term continuous low-dose exposure in both occupational and environmental settings [12,13].

It is well known that some reactive metabolites of benzene can play a critical role in damaging the health of human beings [15,23]. Several studies suggest that bioactivation of benzene produces reactive oxygen species (ROS), which can cause DNA damage and alter cell signal transduction, leading to carcinogenesis [31,38,41].

Although it is well known that excessive ROS production can cause toxicity through oxidative damage to key cellular components [8], interference with normal signalling pathways also has deleterious consequences [32]. In this complex scenario, signalling pathways involving oxidative stress, such as NF-κB, p38-MAPK (mitogen-activated protein kinase), stress-activated protein kinase/Jun. amino-terminal kinase (SAPK/JNK) and signal transducer and activator of transcription 3 (STAT3), are important for various biological processes, including regulation of cell proliferation, differentiation and apoptosis. Given its possible involvement in leukaemogenesis [3,16,26,28], it is hypothesized that chronic exposure to even low-dose benzene can be toxic due to its action on these pathways.

In this study, the effects of low-dose, long-term benzene exposure on NF-κB, STAT3, p38-MAPK and SAPK/JNK signal transduction pathways were evaluated in circulating mononuclear cells in a group of gasoline station attendants. In addition, the influence of consumption of vegetables and fruits (as a rich source of exogenous antioxidants) on these pathways has also been evaluated, as there is increasing evidence of possible interaction of dietary antioxidants with endogenous sources of pro- and antioxidants, thereby increasing the risk of cancer [21,34].

2. Material and methods

2.1. Study population

A total of 91 men, employed in various gasoline stations located in eastern Sicily, were enrolled for this study and compared with a control group of 63 male office workers with no history of benzene exposure. The workers were enrolled in a health surveillance programme to prevent occupational diseases. A written consent was obtained from all participants of this study. A custom-made questionnaire was used to obtain information on their sociodemographic characteristics (e.g. age and body mass index (BMI)), lifestyle (e.g. smoking habit, alcohol consumption and intake of fruits and vegetables) and occupational features (e.g. lifetime exposure to benzene and use of personal protective equipment), and to exclude individuals with known disorders or diseases (e.g. job-related diseases, infection or other pathology involving oxidative stress) in the 3 months preceding the survey.

2.2. Assessment of benzene exposure

Urine samples of participants from 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 trans,trans-muconic acid (t,t-MA) concentration, as a biomarker of benzene exposure, was determined by solid-phase extraction, followed by high-performance liquid chromatography (HPLC) with diode array detection using an Agilent 1200 series HPLC system and a kit produced by Eureka Lab Division (Ancona, Italy). The t,t-MA levels were expressed as micrograms per millilitre.

2.3. Evaluation of signal transduction pathways

Whole peripheral blood samples were collected in tubes containing potassium ethylenediaminetetraacetic acid (K-EDTA) and processed for isolating peripheral blood mononuclear cells (PBMCs) by density gradient centrifugation using Lympholyte® (Cedarlane Laboratories, Burlington, Canada).

Quantitative analyses were performed for proteins NF-κB p65, phospho-NF-κB p65 (Ser536), phospho-SAPK/JNK (Thr183/Tyr185), phospho-p38-MAPK (Thr180/Tyr182), phospho-STAT3 (Tyr705) and phospho-IκB-α (Ser32) using PathScan® Inflammation Multi-Target Sandwich enzyme-linked immunosorbent assay (ELISA) Kit #7276 (Cell Signaling Technology Inc., USA) in accordance with the manufacturer's protocol. Absorbance of samples was read at $\lambda = 450$ nm using a Synergy HT Microplate Reader (BioTek Instruments Inc., Winooski, USA). The assay was performed in technical duplicates.

2.4. Statistical analysis

Data were analyzed by GraphPad Prism software using Student's *t*-test to compare benzene-exposed individuals with those of control group, whereas correlation analysis was performed using Spearman's test with statistical significance set at $p < 0.05$.

3. Results

Sociodemographic characteristics and lifestyle of the study population are presented in Table 1. No signs or symptoms of job-related diseases, infection or other pathology were observed in any of the participants. Most of them had an adequate daily intake of food rich in antioxidants [36,43], did not smoke and did not abuse alcohol. The consumption of one or two glasses of wine or beer every day or up to three servings of liquor per week was considered normal [36].

All participants declared to use adequate personal protective equipment.

Significantly higher t,t-MA levels (Fig. 1, $p < 0.05$) were observed in gasoline station attendants (0.89 ± 0.57 μg/ml, mean ± SD) than controls (0.67 ± 0.45 μg/ml, mean ± SD). Similarly, higher levels of NF-κB and phospho-IκB-α proteins (137.6% and 147.9%, respectively, $p < 0.001$) were observed in PBMCs of benzene-exposed individuals than controls. A significant decrease (32.4%, $p < 0.001$) in phosphorylated STAT3 was observed in gasoline station attendants compared with the control group, and phosphorylated p38 and SAPK/JNK protein levels showed only a slight increase in the exposed group (Fig. 1).

All protein concentrations in PBMCs, except phospho-p38, were positively and significantly correlated with t,t-MA levels (Table 2). Consumption of fruits and vegetables seemed to be significantly associated ($p < 0.05$) only with phospho-NF-κB p65 (Table 2).

No correlation was found between signalling pathway markers in PBMCs and age, BMI or tobacco use.

Table 1
Sociodemographic characteristics and lifestyle of study population. Student's *t*-test did not indicate any significant difference (NS) between benzene-exposed and control groups.

	Benzene-exposed workers	Controls	*p*
N	91	63	NS
Age (years, mean ± SD)	38.13 ± 9.46	40.70 ± 11.39	NS
BMI (mean ± SD)	24.47 ± 2.28	25.22 ± 0.31	NS
Lifetime exposure to benzene (years, mean ± SD)	14.06 ± 6.23	-	NS
Smokers	9 (9.9%)	8 (12.7%)	NS
Alcohol abuse	0	0	
Fruits and vegetables intake (servings/day)			
0	0	0	
1–2	11 (12.1%)	7 (11.1%)	
2–3	35 (38.4%)	26 (41.3%)	
3–4	36 (39.6%)	22 (34.9%)	
≥4	9 (9.9%)	8 (12.7%)	