Benzene and cortisol: relationship in workers exposed to outdoor pollution

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Abstract

***Introduction*: The purpose of our study is to assess whether individual exposure to low-dose benzene in urban air may affect the plasma concentrations of cortisol in a population of outdoor workers in a large Italian city.**
***Materials and methods*: From a population of 1594 workers, 100 outdoor workers were selected. For each worker, a blood sampling was performed to measure benzene and cortisol. Kolmogorov-Smirnov test was used to verify the normality of distribution of the different variables. On the total sample, student T tests were performed for 2 mode variables (sex, smoke and job) and ANOVA test for variables with more than 2 modes (age and seniority). Pearson's correlation index between the variables in the total sample and after division on the basis of sex, smoking habit and manners was evaluated. Were considered significant results with p values below 0.05.**
***Results*: Our study did not show statistically significant correlations between blood benzene levels and cortisol in none of the groups studied.**
***Discussion*: Further studies are needed to confirm the existence of possible significant association between occupational exposure to benzene and cortisol levels alteration.**

KEY WORDS: benzene, biological monitoring, cortisol, environmental pollutants, outdoor workers.

Introduction

Benzene is a volatile, colorless and odorous liquid. It is characterized by a high chemical stability which is the basis of most of the properties of this compound: highly flammable, antiknock, low viscosity, high solubility in polar solvents and low solubility in water. It is rarely present in nature in high quantities, except in oil or during combustion processes such as volcanic eruptions or fire.
The main sources of exposure to the general population are cigarette smoke and air contaminated by high levels of benzene due to vehicular traffic or near petrol stations (1, 2).
The main sources of occupational origin, however, derive from the following uses of benzene: additive in fuels, industrial synthesis of organic compounds such as ethylbenzene (precursor of styrene, used to produce polymers, copolymers, plastic and latex resins), cumene (converted to phenol for the production of adhesives and resins), cyclohexane (used in the production of certain types of nylon), nitrobenzene (for the production of aniline, paints and other products), alkylbenzene (for the production of detergents) and chlorobenzene (polymers, pesticides, paints) (2).
Benzene is responsible for various toxic effects on human health, both acute and chronic (3-5). There is sufficient evidence that benzene is carcinogenic to humans and, in particular, it can cause acute myeloid leukemia and acute non-lymphocytic leukemia. Benzene exposure was also positively associated with acute lymphocytic leukemia, chronic lymphocytic leukemia, multiple myeloma, non-Hodgkin lymphoma (6).
Cortisol is a steroid hormone produced by the adrenal glands, particularly involved in the body's response to physical and psychic stress. In fact, cortisol can act on

almost all cells of the body, promoting the correct addition of fatty acids and glucose and optimizing energy metabolism in organs such as the heart, lungs and muscles. In addition to metabolism regulation, cortisol has important effects on the immune system, promoting anti-inflammatory reactions. In stressful situations, cortisol also helps maintaining proper blood pressure and normal nervous and cerebral activity. Circulating cortisol is predominantly inactive, or linked to plasma proteins. Only a small percentage of the hormone is active at cellular level and in this form is also present in saliva.

Cortisol secretion follows a 24-hour circadian rhythm and has a peak in the morning until it reaches the lowest values around 10-12 PM. Its regulation is based on corticotropin, a hormone secreted by hypophysis: a reduction in the level of cortisol in the blood results in an increase in corticotropin secretion which in turn stimulates the adrenal cortical with a consequent increase in the cortisol rate in blood.

The cortisol role in mediating stress response is well known, but several studies in literature indicate that many physical and chemical pollutants can play an important role by altering hormone levels (7, 8). In addition, cortisol levels may also be influenced by several other factors (9).

Ethanol, for example, can affect glucocorticoid levels; the ingestion of moderate amounts of ethanol causes an increase in cortisol concentration. In addition, high levels of cortisol are found in subjects with chronic ethylosis, both during alcoholic intoxication and during abstinence (10-12).

Cigarette smoking is associated with an increase in cortisol levels, in fact Steptoe and Ussher (13) have shown that smokers have higher levels of this hormone than non-smokers.

Certain studies on animals and humans showed an increase in cortisol levels due to the exposure to physical environmental pollutants such as noise (14) or chemicals such as benzene, toluene (15), carbon monoxide and manganese (16, 17), while vibrations seem to cause a decrease in hormone levels (18).

Several Authors studied plasma cortisol levels in workers exposed to outdoor pollution (19-21), hypothesizing a relationship between hormone levels and outdoor pollution.

The purpose of our study is to assess whether individual exposure to low-dose benzene in urban air may affect the plasma concentrations of cortisol in a population of outdoor workers in a large Italian city.

Materials and methods

Population studied

Workers who performed outdoor tasks and professionally exposed to urban polluters were selected from an initial population of 1594 municipal policemen of Rome. Workers have been randomly identified from 8 different areas of the city (considered to be representative of traffic) and a similar group of workers (35 subjects per area) was taken from each of them for a total of 280 workers, in order to reduce confusion bias.

For the inclusion in the study and in order to identify the confounding factors, a physician administered a clinical-anamnestic questionnaire to each participant to investigate age, residence area over the last 5 years, physiological history (especially focused on cigarette smoking and dietary habits), current and recent history of work, remote and near pathological history (with particular regard to the possible presence of pathologies in the pituitary-adrenal axis and more generally of the endocrine system) and information about the possible extra-working exposure to benzene.

Workers who have been resident for at least five years in the same urban area, where they also worked and were similar to dietary habits and water consumption, were included. Furthermore, the studied population lived in homogeneous (Mediterranean country) environments for furnishings, dwelling types, carpets, etc. where the release of benzene is low and negligible (2).

Roadmill workers were assigned to traffic control in roads and areas with high and medium rate of traffic, monitoring and traffic control at crossroads, parking areas and restricted traffic areas; their job was mainly on foot (22). Other policemen were assigned to traffic control and to intervene in specific cases such as road accidents and other activities requesting the driving of a car or of a motorcycle, as a driver or "second in a patrol". For other outdoor activities, workers had different roles such as Environmental Police and External Commercial Activities (22). Most of these activities were carried out in outdoor environments, while drivers stayed in the car for at least 80% of working time (7 hours a day for 5 days a week).

All workers normally work in the morning and they were monitored during the morning shift (07:00 AM - 02:00 PM) at the end of the working week.

With regard to cigarette smoke exposure, we have taken into account the World Health Organization (WHO) classification, considering smokers like all the people who had already reported having smoked at least 100 cigarettes in their lives, currently smoking or having stopped smoking for less than six months. All workers who stated that they had stopped smoking for more than 6 months were considered non-smokers (WHO, 2014).

In order to avoid the influence of confounding factors, we have excluded from the study the workers exposed to solvents, lubricants, detergents, etc. during non-working activities (23, 24), drug users and alcoholic drinkers (alcohol consumption more than 2 alcoholic units per day for men and 1 alcoholic unit for women; 1 alcoholic unit is about 12 grams of ethanol) (25, 26), subjects with pre-existing or ongoing endocrine disorders (adeno-physiological pathologies, adrenal pathologies). Workers who used (in the six months prior to the study) drugs that can alter the levels of cortisol (e. g. corticosteroids, estroprogestin, potassium-sparing diuretics, metoclopramide) (27-30) and who carried out outdoor tasks for less than 1 year, were also excluded.

The final sample of the study included 100 workers (77 men and 23 women, 28 smokers and 72 non-smokers), divided according to the job of 62 road-blocks and 38 workers with other outdoor tasks (e. g. drivers).

The characteristics of the study population are shown in Table 1.

The division of smokers and non-smokers has proved to be necessary since cigarette smoking has been shown to be associated with an increase in cortisol levels (13). All subjects were informed that the research protocol data would be treated in an anonymous and collective manner, using scientific methods and for scientific purposes, according to the principles of the Helsinki Declaration.

Environmental monitoring: personal dosimetry

Eight personal dosimetry, selected from 8 workstations considered representative of the topographic distribution of the workers studied, as well as 4 representative personal dosimeters for drivers in service cars in with at least 2 agents for each shift (so even though only one worker wore the dosimeter, the results were considered representative of the colleague's exposure, because he was in the car with him). The sampling lasted for the whole working shift. Air and blood samples were collected in parallel in the study participants to avoid the influence of weather and seasonal conditions on personal exposure to air benzene.

Workers have been equipped with passive personal dosimeters, attached as badges to the worker's neck at the beginning of each sampling day, in order to measure the concentration of benzene in the breathing area.

Table 1 - Characteristics of the study population divided for task.

Variables	Traffic policemen	Policemen with other outdoor activities
Number of subjects	(n.62)	(n.38)
Gender	49 (79)	27 (71)
Male n° (%)	13 (21)	11 (29)
Female n° (%)		
Smoking habit	15 (24,2)	13 (34,2)
N° (%)		
Age (ys)	46,83 (9,85)	42,94 (7,73)
Mean (SD)	45,78	42,25
Geometric Mean	29-64	28-60
Min-Max	46,5	42
Median		
Working life (ys)	18,09 (10,82)	14,76 (7,01)
Mean (SD)	14,79	13,29
Geometric Mean	6-37	6-35
Min-Max	17	16,5
Median		
Benzene		377,84 (470,87)
Mean (SD)	360,71 (319,40)	216,70
Geometric Mean	236,16	75-1926
Min-Max	75-1338	184
Median	246	
Cortisol		148,85 (49,73)
Mean (SD)	155,35 (50,17)	140,32
Geometric Mean	144	62-259
Min-Max	34-362	143
Median	153	
Air benzene	8	4
Number of subjects	17,28 (10,42)	10,81 (7,85)
Mean (SD)	13,88	6,63
Geometric Mean	5-36,6	1-24,8
Min-Max	16,2	11,2
Median		

After sampling, the passive dosimeters were prepared for desorption by adding CS^2 (carbon sulphide) to the badges. Benzene analysis was performed by capillary gas chromatography (GC) with the "Dani gas-chromatograph 1000" equipped with a flame ionization detector (FID) for quantification.
The detection limit for benzene (LoD) was 0.001 ppm (3.19 μg/m^3). The observed concentrations of benzene were expressed as mean weighted values over a period of 8 hours.

Dosage of cortisol and blood benzene
For each worker, the blood draw for the determination of blood benzene was performed after 5 continuous working days at the end of the working shift during the same day of personal benzene sampling in the air. Blood benzene monitoring was performed after the distribution of an informative note containing the recommendation to abstain from the active and passive smoking, the consumption of certain foods (offal, chicken, fish and nuts) and the intake of alcoholic or super alcoholic beverages (2, 25).
All workers included in the study were subjected to cortisol dosage via RIA (radioimmunoassay): from each worker a sample of 10 ml of venous blood was taken at 08.00 AM o'clock at fasting. Blood samples were stored in the refrigerator at -4°C until the time they were transferred (in a suitable container and at the same temperature) to the laboratory where they were centrifuged immediately and subsequently stored at -20°C until analyzed (within 3 days).
The normal ranges used in laboratory analysis were 50-230 ng/ml (cortisol values at 08:00 AM).
The laboratory performed the tests by the extraction method with SPME technique (extraction with ethyl acetate and esterification) and gas chromatography with mass spectrometry detector (GC-MS) with a detection limit of <150 ng/L (28).

Statistic analysis
For the statistical evaluation, the following factors were considered: sex, habit of smoking cigarettes, job, age and seniority. The normality of the distribution of the different variables was verified by the Kolmogorov-Smirnov test, which was statistically significant for both cortisol and blood benzene; then the parameters analyzed were in a non-normal distribution and so they were converted into logarithmic form.
The results of the environmental monitoring (atmospheric benzene), biological monitoring (blood benzene) and cortisol were expressed in terms of mean, standard deviation (SD), median and range (min-max). The concentrations below the LoD were replaced by the middle of LoD.
The comparison of the averages was done by Student's T-test for independent variables for 2-mode variables (sex, cigarette smoking habit and working job) and ANOVA for variables with more than 2 modes (age, seniority).
In the total sample and after the subdivision according to sex, smoking, and occupational activity, Pearson correlation index was calculated to verify the level of association between blood benzene values and cortisol values.
The results were considered significant when p values were less than 0.05. The statistical analysis was done using the SPSS® Advanced StatisticalTM 24.0 software.

Results

Environmental monitoring of benzene: personal dosimetry
Individual benzene exposure values in the air are shown in Table 1. None of the subjects smoked for the previous 5 days and during the sampling period. No sample exceeded the limit value of 1.6 mg/m^3 of benzene, as proposed by the American Conference of Governmental Industrial Hygienists (16) for subjects with professional exposure.
Dosimetry also showed atmospheric benzene values above 5 μg/m^3 (in Italy, this is the limit value for human health protection, laid down by Ministerial Decree 60/2002, based on Directive 2000/69/EC), for 6 roadblocks on 8 and for 2 drivers on 4.

Cortisol, blood benzene and characteristics of the studied population
The characteristics of the studied population, blood and atmospheric levels of benzene and cortisol, are shown in Table 1. A statistically significant difference between the cortisol and the smoking habit was observed by Student's T test while a non statistically significant relationship with sex and work; even with regard to blood benzene parameters, no statistically significant difference was observed either with respect to sex or with respect to cigarette smoke and working life.

Blood benzene and cortisol
Pearson correlation analysis between blood benzene levels and plasma cortisol values did not indicate the existence of statistically significant correlation in both the total sample and all subgroups (Table 2).

Discussion

In literature it has been found that the exposure to urban physical and chemical stress, interacting with psychosocial factors and lifestyle, may have an influence on cortisol levels in road policemen (19). There is also evidence that benzene in the air affects both the hormonal system (22, 29) and other organs and apparatuses (31-33). Other studies, finally, observed the action on the neuroendocrine system, *in vivo* and *in vitro*, of the exposure to other hydrocarbons and organic solvents (34).
Our study did not show statistically significant correlations between blood benzene levels and cortisol in none of the groups studied.

Table 2 - Pearson correlation coefficient (R) between log cortisol values and log total blood benzene in the total sample and after subdivision on the basis of sex, cigarette smoking and kind of task.

Variables	Biological indicator (Mean ± SD)	Cortisol
Total sample	302.76 ± 297.55	r: -,312 [a] p: 0,000 [b]
Male subjects	333.17 ± 337.9	r: -,532 [a] p: 0,000 [b]
Female	249.34 ± 194.6	r: -,607 [a] p: 0,000 [b]
Smoker subjects	332.03 ± 372.31	r: -,492 [a] p: 0,000 [b]
Non smoker subjects	290.35 ± 259.95	r: -,382 [a] p: 0,000 [b]
Police drivers	337.58 ± 398.8	r: -,208 [a] p: 0,097 [b]
Policemen with other outdoor activities	183.44 ± 46.32	r: -,754 [a] p: 0,000 [b]

For this reason, this must be considered a preliminary study; it is not possible to assert with certainty that there is no correlation between the variables studied, since the sample considered is quite small, although some evidence indicates the correlation between urban pollutants and cortisol (19, 20). Therefore, our results induce us to expand the studied population, deepening the study on the possible correlation between benzene levels in the air and plasma cortisol levels.

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