Plasma 17- α -OH-progesterone in Male Workers Exposed to Traffic Pollutants

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Abstract: The aim of the study is to evaluate if the occupational exposure to urban pollutants could cause alterations on 17- α -hydroxy-progesterone plasma levels and related diseases in male traffic policemen. 17- α -OH-P is synthesized in Leydig cells and in adrenals; it influences spermiogenesis, acrosoma reaction, testosterone biosynthesis, blocking of gonadotropin secretion; it regulates learning, memory and sleep. After excluding principal confounding factors, i.e., rotating or night shifts, exposure to solvents, paints and pesticides during time-off and smoking, traffic policemen were matched with controls by age, working life and drinking habit. Finally, 112 traffic policemen and 112 controls were included in the study. In traffic policemen 17- α -OH-P mean values were significantly higher vs. controls. The distribution of 17- α -OH-P values in both groups was significant. An increased frequency of fertility disorders referred to the questionnaire items were found in traffic policemen vs. controls, but the difference was not significant. The occupational exposure to low doses of chemical urban stressor, interacting with and adding to the psychosocial ones, could alter plasma 17- α -OH-P concentrations in traffic policemen vs. controls. 17- α -OH-P could be used as an early biological marker, even before the onset of the reproductive and mental health diseases.

Key words: Traffic policemen, Stressor, Traffic pollutants, Plasma 17- α -hydroxy-progesterone, Male reproductive health

Introduction

During exposure to stress stimuli, the human body responds physiologically by increasing the activity of both the hypothalamic-pituitary-adrenal (HPA) axis and the sympathoadrenal system (SAS). Throughout life, the human organism is constantly exposed to various stressors, and its ability to respond to these stressors directly affects a person's overall health. Stress is proven to be a major cause of illness and reduced productivity, especially in occupational setting.

Workers operating in urban environment, such as traffic policemen, are daily exposed to chemical stressor¹⁾ that

interact with and adding to psychosocial ones^{2,3)}: for these workers, the working environment corresponds to the life environment of the general population.

In our previous studies, carried out on traffic policemen, we also observed, besides the well known effects on the cardiovascular and respiratory systems⁴⁻⁶⁾, the effects on the neuro-immune-endocrine system, including the HPA-axis and related systems. The results of these studies allow us to suggest that the alterations found in the tested parameters, including growth hormone (GH), insulin-like factor type 1 (IGF-1), adrenocorticotrophic hormone (ACTH), 5-hydroxyindolacetic acid (5-HIAA), cortisol (CORT), vasopressin (VP), homovanillic acid (HVA), insulin, response of lympho-monocytes to phytohemagglutinin, serum levels

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of IgG anti Herpes Simplex Virus Type 1 (IgG anti-HSV-1), could be indicative of the early neuro-immune-endocrine system response to chemical stressor⁷⁻¹⁶). Therefore, these parameters could be considered as early biological markers of chronic exposure to urban stressor. Moreover, it is well known that personal exposure to pollutants from direct exposure to traffic fumes, as experienced by some categories of outdoor workers, such as traffic policemen, may be considered higher than personal exposure of indoor workers (particularly in Italian cities)¹⁷). For this reason in these workers we have already monitored in previous researches the following parameters: urinary S-phenylmercapturic acid, trans-trans muconic acid, pyrenol acid; environmental and blood benzene; serum nickel (Ni); sister chromatid exchanges (SCE) and micronuclei (MN) in peripheral blood lymphocytes; and antibodies to the benzo(a)pyrene diol epoxide-DNA adducts in sera. In particular, we found that exposure dosage to benzene (time weight average, TWA) (mean 10.7 and 3.6 $\mu\text{g}/\text{m}^3$, respectively) and toluene (mean 40.7 and 13.5 $\mu\text{g}/\text{m}^3$, respectively) was significantly higher among traffic policemen than among indoor workers¹⁸⁻²⁴). Since in these previous studies we had already measured the environmental and biological levels of principal pollutants in our working population and it is well known that traffic policemen's dosage is significantly higher than controls, we didn't repeat the exposure dosage study in this work. Furthermore during the period March-April 2004, in the city of this study, mean value in air of benzo(a)pyrene (BaP) (0.48 ng/m^3), polycyclic aromatics hydrocarbons (PAHs) (4.45 ng/m^3), lead (Pb) (22 ng/m^3), Ni (9 ng/m^3), carbon monoxide (CO) (1.4 mg/m^3), nitrogen dioxide (NO_2) (77.5 $\mu\text{g}/\text{m}^3$), ozone (O_3) (49.4 $\mu\text{g}/\text{m}^3$), benzene (4.35 $\mu\text{g}/\text{m}^3$), particulate matter, 10 μm in diameter or less, (PM_{10}) (50 $\mu\text{g}/\text{m}^3$) and sulphur dioxide (SO_2) (4.7 ng/m^3) have been monitored²⁵).

Studies in literature suggest that exposure to urban chemical stressor such as lead (Pb)²⁶⁻³⁰), cadmium³¹), diesel exhaust³²), mercury (Hg)³³) and styrene³⁴) could cause decrease or increase in synthesis, secretion and/or action of 17- α -hydroxy-progesterone (17- α -OH-P) or cause male reproductive health disorders. Other studies have pointed out the possibility of developing reproductive health diseases, in men exposed to urban chemical pollutants, such as sulphur oxide (SO), Pb^{35, 36}), NO_2 and nitrogen oxide (NO)^{37, 38}).

Besides these above mentioned chemical agents, traffic policemen are also exposed to psycho-social stressor. Our previous research has ascertained through the compilation and elaboration of a questionnaire, a greater subjective stress in traffic policemen compared with a control group. Sources

of psychosocial stressor for the traffic policemen may be the relation with the public, exposure to episodes of criminality, and the need to maintain high levels of services in various contexts³⁹). Also psycho-social stressor may affect reproductive health⁴⁰).

In men 17- α -OH-P is synthesized not only in the Leydig cells of testicles, but also in the adrenal glands⁴¹). 17- α -OH-P has direct effects upon the process of spermatogenesis⁴²⁻⁴⁵) and on the Central Neural System (SNC) where it acts as a positive or a negative modulator of GABA A receptor (γ -aminobutyric-acid_A)⁴⁶); moreover, it regulates several activities, such as learning, memory⁴⁷) and sleep⁴⁸). Furthermore, according to other studies alterations in plasma 17- α -OH-P levels could cause mental health disorders in male subjects, for instance: panic attacks, anxiety, post traumatic stress disorders, depression and schizophrenia⁴⁹⁻⁵²).

The aim of the study is to evaluate if the occupational exposure to urban pollutants could cause alterations on 17- α -OH-P plasma levels and related diseases in traffic policemen compared to a control group.

Materials and Methods

The research was carried out on a working population of 503 male Municipal Police employees (299 traffic policemen and 204 controls). For the inclusion into the study a questionnaire with the physiological anamnesis, the remote and near pathological anamnesis and the previous and current working history, including also the confounding factors reported below was collected for all workers, in presence of a physician. The questionnaire comprised as well, items referred to fertility and mental health disorders collected according to a binary method (yes/no). The mental health disorders investigated were at the time of interview. To this aim has been used the following item: "do you actually have mental health disorders? If yes describe them".

In order to avoid the influence of confounding factors, who referred performing rotating or night shifts, being exposed to solvents, paints and pesticides during time-off, and current smokers⁵³) were excluded from the study.

We divided the remaining subjects into traffic policemen and controls. The traffic policemen exposed to urban pollutants worked on parking control, control of access openings to limited-traffic areas, and control of crossroads or roads with heavy traffic. Subjects with indoor activities, (i.e. administrative and bureaucratic duties) with lower levels of exposure, were used as control group.

Both groups worked 7 h/d for at least 5 d/wk.

Traffic policemen were matched with controls (by mean, standard deviation and distribution into classes) by age, working life and drinking habit (number of glasses of wine/beer per day and number of glasses of spirits per week)⁵⁴. In total, 224 subjects were included in the study: 112 traffic policemen and 112 controls.

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

A 10 ml sample of venous blood was taken from each worker between 8 and 10 in the morning, before they had eaten. Samples were kept in the workplace in a refrigerator at -4°C until they were transferred (by means of a container and at the same temperature) to the laboratory, where they were immediately centrifuged and the serum was stored at -20°C until analysis (within 3 d).

The samples were taken during the period March 2004–April 2004.

The laboratory executed the 17- α -OH-P dosage on venous blood samples through the analytical method RIA (radio-immuno-assay). Our laboratory normal values for male sex were between 0.61–3.34 ng/ml.

The laboratory did not know which samples came from the group of traffic policemen and which from the control group, although both the physicians in charge and the technicians knew a study was in progress.

All subjects agreed to the processing of their personal data, declared being aware that they came within the category of “sensitive data”, and consented that the ones arising from the research protocol should be treated in an anonymous and collective way, with scientific methods and for scientific purposes in accordance with the principles of Helsinki Declaration.

For the methodological organization of data collection, specific socio-communicative competences related to the development of tools suitable for the collection of information and analysis data were used.

Statistical analysis

Statistical analysis of the data was based on the calculation of the mean, standard deviation (SD), distribution, the minimal and maximal values and frequency according to the nature of the single variables. The differences between the means were compared using Student's *t*-test for the unpaired data. The frequencies of the single variables were compared using the chi-square test with Yates' correction. The differences were considered significant where the *p* values were <0.05 . The statistical analysis has been done using the statistical program Solo-BMDPTM Statistical Software.

Table 1. Mean age, mean working life, mean drinking habit and mean 17- α -OH-plasma levels in population studied

	Traffic policemen N° 112	Controls N° 112
Age (yr)	41.9 (4.4)	42.8 (4.3)
Mean (SD)	35–50	31–50
Min-Max		
Working life (yr)	8.6 (5.5)	7.7 (5.8)
Mean (SD)	1–26	1–28
Min-Max		
Drinking habit		
(wine, beer: n. glasses/day)	0.72 (0.88)	0.86 (0.94)
Mean (SD)	0–1	0–1
Min-Max		
Drinking habit		
(spirits: n. glasses/week)	1.1 (0.5)	1.2 (0.4)
Mean (SD)	0–1	0–1
Min-Max		
17- α -OH-P (ng/ml)	1.7 (0.5)*	1.2 (0.3)
Mean (SD)	1.1–3.8	0.5–2.1
Min-Max		

SD = standard deviation; yr=year; **p*=0.000 in traffic policemen vs. controls.

Results

In traffic policemen 17- α -OH-P mean values were significantly higher compared to controls (*p*=0.000) (Table 1). The distribution of 17- α -OH-P values in traffic policemen and controls was significant (*p*=0.000). The number of traffic policemen with 17- α -OH-P values outside the lower and upper normal limit of our laboratory was not significant compared to controls (none traffic policemen vs. n. 2 controls and n. 1 traffic policemen vs. none controls respectively).

Individual and mean 17- α -OH-P values in male traffic policemen and controls are shown in figure 1.

We have found a little correlation (*r*) between 17- α -OH-P levels and length of service (*r* = 0.20) for both traffic policemen and controls.

An increased frequency of fertility disorders referred to the questionnaire items were found in traffic policemen compared to controls, but the difference was not significant (3.7% vs. 0.9% respectively in traffic policemen and controls; *p*>0.05).

No significant differences were found in percentage of traffic policemen compared to controls, in reference to questionnaire items concerning mental health diseases (depression, anxiety, panic attacks) (4.6% vs. 3.7% respectively in traffic policemen and controls; *p*> 0.05).

Fig. 1. Individual and mean 17- α -OH-P values in male traffic policemen and controls.

Discussion

Few studies in literature have focused on the occupational exposure to chemical urban pollutants and effects on male reproductive health. Studies in literature showed that exposure to chemical stressor, present also as urban pollutants, can cause 17- α -OH-P alterations in male sex. For example in animals and humans, exposure to Pb affects biosynthesis of steroid hormones from Leydig cells, and causes a decrease in 17- α -OH-P levels^{26, 27, 30}. Moreover in a study on animals, have evaluated the effects of Cd on circulating steroids; the authors observed a reduction of testosterone (T) and an increase of progesterone synthesis, indicating a time/dose depending effect of Cd upon the above mentioned hormones³¹. An increase in mean plasma 17- α -OH-P levels by studying the effects of octylphenol and bisphenol, present in diesel exhaust on this hormone were observed³².

The above mentioned data are in accordance with the results obtained from our study: in traffic policemen we observed a statistically significant increase of 17- α -OH-P compared to controls. We can deduce that this result could be due to a chronic exposure to low doses of chemical urban stressor, present in the urban environment. Furthermore we found a little correlation ($r=0.2$) for both traffic policemen and controls. This result suggests that 17- α -OH-P levels start increasing after a "brief" occupational exposure period. In the long run, the increased level and duration of exposure could induce decreased plasma 17- α -OH-P values, a biphasic dose/response relationship which is similar to the hormesis phenomenon⁵⁵. In this phenomenon a chemical exerts opposite effects depending on exposure dosage: there is a

biological activation at low doses, but an inhibition at high doses, resulting in a U or inverted U-shaped dose response.

Still, since the values of 17- α -OH-P found outside the normality range, for both traffic policemen and controls, were not significant, we can deduce that the above mentioned results can be valuable for the group, but not for the individual.

Chemical urban stressor present also in urban air can alter male reproductive function. Exposure to Pb and Cd determines a possible damage in the testicular function and a reduction in sperm production^{28, 29}. Exposure to Hg has been associated with an increased incidence of cryptorchidism and hypospadias in newborns whom mothers had previously been exposed to Hg and a diminution of sperm density and a major risk of developing testicular cancer in adult men³³. A study has shown that the exposure to styrene determines denaturation of DNA and reduction in sperm density³⁴.

A group of authors, have evaluated the fertility of 85 men, professionally exposed to urban pollutants by studying the quality of their semen and the plasma levels of sexual hormones and of methaemoglobin, sulphaemoglobin, carboxyhaemoglobin, Pb and zinc-protoporphyrin. The results demonstrated an inverse correlation between quality of semen and plasma concentrations of methaemoglobin and Pb, which could indicate a negative influence of NO and Pb on sperm parameters in workers exposed compared to a control group³⁵. In workers exposed to Pb was also observed a lengthening of time to pregnancy (TTP)³⁶. A study conducted upon male, sexually mature laboratory animals exposed to diesel exhaust, containing NO₂ and PM₁₀ has shown a decrease in sperm production due to a noticeable damage of Sertoli cells and a reduction of their number³⁷. In a previous study, it had already been observed an analogous

reduction of spermatogenesis in rats exposed to diesel exhaust in which had also been registered a depression of pituitary gonadotropins secretion³⁸⁾.

Furthermore, several studies have pointed out that 17- α -OH-P affects some neurological functions; through the activation of GABA_A receptor, which exert an inhibitory function on the SNC⁴⁶⁾. Progesterone and its metabolites can induce psychological disorders in men, such as panic attacks⁴⁹⁾, mood disorders (depression and post-traumatic stress disorders)⁵⁰⁾ and anxiety⁵¹⁾. High levels of 17- α -OH-P have also been reported in schizophrenic men⁵²⁾.

In our study, in reference to the questionnaire items, an increased frequency of fertility disorders and mental health diseases was not observed, in traffic policemen compared to controls. That is why the screening of plasma 17- α -OH-P levels could represent a useful biological marker of exposure to chemical urban pollutants.

In conclusion, considering that the subjects with the principal confounding factors were excluded from the study and that traffic policemen and controls were matched for the variables previously mentioned, our results suggest that the occupational exposure to low doses of chemical urban stressor, interacting with and adding to the psychosocial ones, could alter plasma 17- α -OH-P concentrations in traffic policemen of male sex compared to a control group. In this study we don't measure directly chemical pollutants in the population studies and therefore the results should be interpreted with the caution and conservative tone. According to our previous researches on other neuro-immune-endocrine parameters⁷⁻¹⁶⁾, 17- α -OH-P could be used as an early biological marker, valuable for the group, to be employed in occupational sets.

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