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Short communication

Sputum induced cellularity in a group of traffic policemen

Silvano Dragonieri [a], Marina Musti [b], Carmelina Izzo [c], Luisa Maria Esposito [c], Maria Pia Foschino Barbaro [d], Onofrio Resta [a], Antonio Spanevello [c,d,*]

[a] *Department of Respiratory Diseases, University of Bari, Bari, Italy*
[b] *Insitute of Occupational Diseases, University of Bari, Bari, Italy*
[c] *Fondazione Salvatore Maugeri, Care and Research Institute, Cassano delle Murge (Bari), Italy*
[d] *Department of Respiratory Diseases, University of Foggia, Foggia, Italy*

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Abstract

It has been demonstrated that a group of workers (e.g. waste handlers) daily exposed to a traffic related air pollution present airway inflammation in term of an increase of neutrophilic inflammation. The aim of our study was to determine the presence of airways inflammation detected by induced sputum in a population of traffic policemen (TP) in the city of Bari, compared to a group of healthy subjects (HS) without any occupational exposure to inhalation of traffic-related air pollution. Twelve non smokers, non atopics, healthy traffic policemen with a history of exposure to airway pollution and 12 HS underwent sputum induction.

TP show a statistically significant increase in the percentage neutrophil cell count (median and IQ range) compared to the HS (65 and 13.5 vs. 40.5 and 9.5; $p<0.01$). In conclusion we have found that policemen chronically exposed to air pollution presented airway neutrophilic inflammation and the results of this pilot study could be strictly considered for the long term effect of a traffic pollution in airway inflammation and the lung function.

Keywords: Occupational exposure; Air pollution; Induced sputum; Airway inflammation; Neutrophil cell

1. Introduction

Air pollution has been generally recognized as a health hazard. Numerous epidemiological studies have shown clear associations between outdoor air pollution and indices of pulmonary and cardiac morbidity within the general population (Health Effects of Outdoor Air Pollution, 1996). An important source of atmospheric pollution is exhaust from car traffic. Health effects specifically associated with traffic-related air pollution have been studied mainly in workers exposed to high concentrations of automobile pollution exhaust fumes that were considerably above threshold values for environmental air. Traffic related air pollution therefore can be considered an occupational health hazard to workers who perform physical labor close to traffic (Raashou-Nielsen et al., 1995; Gamble et al., 1987). It has been demonstrated that a group of workers daily exposed to air pollution present airway inflammation in term of an increase of netrophilic inflammation (Heldal et al., 2003). In particular, traffic policemen are a population group under risk due to their inhalation for several hours/day of particulate matter (PM) rich air. Moreover, some recent

* Corresponding author. Fondazione Salvatore Maugeri, Care and Research Institute, Via per Mercadante km. 2, 70020 Cassano delle Murge (BA), Italy. Tel.: +39 0807814229; fax: +39 0807814272.
E-mail address: aspanevello@fsm.it (A. Spanevello).

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"in vitro" studies indicated release of cellular chemoattractants by components of diesel exhaust particles (Heldal et al., 2003; Bolognesi et al., 1997a; Chandrasekaran et al., 1996).

The aim of our study was to determine the presence of airway inflammation detected by induced sputum in a population of traffic policemen in the city of Bari, compared to a group of healthy subjects without any occupational exposure to inhalation of traffic-related air pollution.

2. Methods

2.1. Study population

The University of Bari was involved in the enrolment of the volunteers (98 subjects over a total of 402 policemen of the City of Bari). Thirty two volunteers satisfied the inclusion criteria: non smokers (lifetime never-smokers), non atopics (negative skin prick tests), no history of asthma or other respiratory symptoms (no subjects had reported symptoms of airway infections in the previous three months), normal lung function (FEV1 higher than 80% predicted), and normal methacholine airway responsiveness (PD20 FEV1> 3200 μg). As a "pilot study" (without a sample size evaluation) 15 policemen with a history of exposure to airway pollution and 17 clerk policemen without any occupational airway exposure were examined for the sputum induction. Three subjects of the group with a history of exposure to airway pollution and five subjects for the group without any occupational airway exposure did not produce an adequate sample for cellular analysis.

2.2. Study design

The subjects were studied for two days within two weeks. On the first day the subjects were documented by means of a questionnaire, skin prick tests to a range of antigens (dog hair, cat fur, *Ambrosie*, *Betulacee*, *Composite*, *Graminacee*, *Parietaria*, *Salicacee*, *Alternaria alternata*, *Oleacee*, *Aspergillus fumigatus*, *Cladosporium*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*). On the second day spirometry, methacholine inhalation tests, and induced sputum were performed. The Ethics Committee of Bari approved the study, and all volunteers gave written informed consent.

2.3. Methacholine challenge

Methacholine (Sigma Chemicals Co., St. Louis, MO) was dissolved in distilled water and delivered by an ampoule-dosimeter device (Mefar, Brescia, Italy) driven by compressed air at a pressure of 1.5 kg/m^2 with 1-s actuation and 5-s intervals between breaths. Aerosols were inhaled during quiet tidal breathing. After inhalation of isotonic saline as a control, doubling doses of methacholine were inhaled from 20 to 3200 μg. A 3-min interval was allowed before each dose increment. FEV_1 was measured 1 min after each dose and the best of three acceptable measurements was retained to create dose–response curves. The non-cumulative doses causing a 20% fall of FEV_1 from control (PD20 FEV1) were calculated by interpolation between two adjacent points of the log dose–response curves. (Crimi et al., 1998)

2.4. Sputum induction

2.4.1. Inhalation procedure

After baseline FEV_1 and FVC measurements, salbutamol was given by inhalation (200 μg by MDI) and subjects inhaled hypertonic (4.5%) saline nebulized for periods of progressively increasing length (1, 2, 4, 8 min). FEV_1 was re-measured 1 min after each inhalation period. An ultrasonic nebulizer (DeVilbiss 65, DeVilbiss Corporation, Somerset, PA, USA), nebulized saline solutions (Spanevello et al., 1998).

2.4.2. Sputum processing

The collected sputum samples were examined within 2 h. Selected portions of the sputum sample originating from the lower respiratory tract were chosen using an inverted microscope and weighed. Dithiothreitol (DTT, Sputolysin, Calbiochem Corp, San Diego, CA, USA), freshly prepared in a dilution of one in 10 with distilled water, was added in a volume (in μl) equal to 4 times the weight of the sputum portion (in mg). Selected sputum was placed in a shaking water bath at 37 °C for 20 min and homogenized. It was further diluted with phosphate

Table 1
Comparison of sputum between Traffic Policemen (TP) and Control group (HS)

	TP	HS	*p*
AGE (years)	40.4±7.9	42.7±7.0	n.s.
FEV1 (%)	98.7±8.4	107.7±13.4	n.s.
FVC (%)	99.3±11.8	103.0±9.7	n.s.
Total cell count×10^5/ml	4.6 (5.2)	4.6 (5.6)	n.s.
Macrophages (%)	32.5 (15.5)	56 (25)	<0.01
Neutrophils (%)	65 (13.5)	40.5 (9.5)	<0.01
Eosinophils (%)	2 (1)	1 (3)	n.s.
Lymphocytes (%)	1 (2)	1 (2)	n.s.

Values of age, FEV1 and FEC are presented as mean±SD. Values of cells are presented as median (IQ range).

buffered saline in a volume equal to the sputum plus DTT. The suspension was filtered through gauze to remove mucus and was centrifuged at 1000 rpm for 5 min. The supernatant was aspirated and frozen at −70 °C for later analysis. The cell pellet was resuspended in a volume of PBS equal to that of the sputum plus DTT and PBS as above. Total cell count (TCC) and viability (Trypan blue exclusion method) were determined using a Burkers chamber haemocytometer. The cell suspension was placed in a Shandon 3 cytocentrifuge (Shandon Southern Instruments, Sewickley, PA, USA) and cytospins were prepared at 450 rpm for 6 min. Cytospin slides were fixed by methanol and were stained by May Grunwald Giemsa for an overall differential cell count on 500 nucleated non-squamous cells. Only samples with cell viability >50% and squamous cell contamination <20% were considered adequate (Spanevello et al., 1998).

2.5. Statistical analysis

Descriptive statistics were used to summarize clinical and demographic characteristic of the subjects. The results were expressed as mean and standard deviation for age and lung function values. The cellular counts were expressed as median and IQ range. The comparison between groups was assessed respectively by two tail t student for age and lung function and Mann–Whitney t test for cellular counts. A value of $p<0.05$ was considered statistically significant.

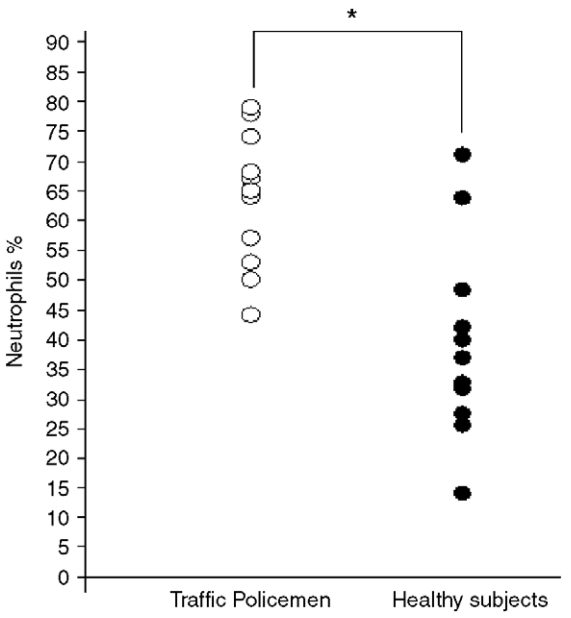

Fig. 1. Neutrophil cell count of induced sputum. The group of traffic policemen (open circles) shows a statistically significant increase of the neutrophils compared to the control group (closed circles) ($p<0.01$). The open circles and the closed circles are 11 (instead of 12) because two subjects present the same value of neutrophils (65% for open circles and 42% for closed circles).

3. Results

The main characteristics of the subjects of both groups with total and differential cell count are shown in Table 1. There are no significant differences in term of age and mean baseline FEV1 and FVC between the two groups. TP show a statistically significant increase in the percentage neutrophil cell count (median and IQ range) compared to the HS (65 and 13.5 vs. 40.5 and 9.5; $p<0.01$) (Fig. 1).

4. Discussion

In this study we determined the presence of airways inflammation detected by induced sputum in a population of traffic policemen in the city of Bari, compared to a group of healthy subjects without any occupational exposure to inhalation of air pollution. On the basis of the results we found that the policemen exposed to traffic related air pollution presented airway neutrophilic inflammation.

Several authors have previously studied the health effects specifically linked to traffic related air pollution in workers like ferrymen, garage and tunnel workers, who are exposed to high concentrations of automobile pollution exhaust fumes that are considerably above threshold values for environmental air (Raashou-Nielsen et al., 1995; Gamble et al., 1987). The originality of this study is the selection of the workers, as to our knowledge no study has evaluated the airway inflammation by induced sputum in a group of traffic policemen. However, Heldal et al. (2003) showed the presence of airway inflammation with an increase of the percentage of neutrophils and IL-8 assessed by induced sputum in a group of waste handlers exposed to bioaereosols. Moreover, the inflammatory response to the traffic exposure by traffic policemen was evaluated with the analysis of respiratory symptoms (Karita et al., 2001), CO level in the expiration air (Atimtay et al., 2000) and blood (Bolognesi et al., 1997b). All these studies were in line with our data of an increase of airway inflammation related to the traffic exposure.

In conclusion we have found that policemen exposed to air pollution presented airway neutrophilic inflammation and the results of this pilot study could be strictly considered for the long term effect of a traffic pollution in the lung function and a larger longitudinal study would

be welcome. Moreover, the quantification of carbon in macrophages and the measurement of IL-8 would give additional and interesting information in the issue.

References

Atimtay AT, Emri S, Bagci T, Demir AU. Urban CO exposure and its health effects on traffic policemen in Ankara. Environ Res Sect A 2000;82:222–30.

Bolognesi C, Gallerani E, Bonatti S, De Ferrari M, Fontana V, Valerio F, et al. Sister chromatid exchange induction in peripheral blood lymphocytes of traffic police workers. Mutat Res 1997a;394:37–44.

Bolognesi C, Merlo F, Rabboni R, Valerio F, Abbondandolo A. Cytogenetic biomonitoring in traffic police workers: micronucleus test in peripheral blood lymphocytes. Environ Mol Mutagen 1997b;30:396–402.

Chandrasekaran R, Samy PI, Murthy PB. Increased sister chromatid exchange (SCE) frequencies in lymphocytes from traffic policemen exposed to automobile exhaust phocytes from traffic policemen exposed to automobile exhaust pollution. Hum Exp Toxicol 1996;15:301–4.

Crimi E, Spanevello A, Neri M, Ind PW, Rossi GA, Brusisco V. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. Am J Respir Crit Care Med 1998;157(1):4–9 [Jan].

Gamble J, Jones W, Minshall S. Epidemiological–enviromental study of diesel bus garage workers. Acute effects of NO2 and respirable particulate on the respiratory system. Environ Res 1987;42: 201–14.

Health effects of outdoor air pollution. Committee of the Environmental and Occupational Health. Assembly of the American Thoracic Society. Am J Respir Crit Care Med 1996;153:3–50.

Heldal KK, Halstensen AS, Thorn J, Eduard W, Halstensen TS. Airway inflammation in waste handlers exposed to bioaerosols assessed by induced sputum. Eur Respir J 2003;21:641–5.

Karita K, Yano E, Jinsart W, Boudong D, Tamura K. Respiratory symptoms and pulmonary function among traffic police in Bangkok, Thailand. Arch Environ Health 2001;56:467–70.

Raashou-Nielsen O, Nielsen ML, Gehl J. Traffic-related air pollution: exposure and health effect in Copenhagen street cleaners and cemetery workers. Arch Environ Health 1995;50:207–13.

Spanevello A, Beghe B, Bianchi A, Migliori GB, Ambrosetti M, Neri M, et al. Comparison of two methods of processing induced sputum: selected versus entire sputum. Am J Respir Crit Care Med 1998;157(2):665–8 [Feb].