Effects of Rome (Italy) winter urban air particles on monocytic macrophagic RAW 264.7 cell line.

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Introduction

Several epidemiological studies have shown statistical associations between exposure to increased particulate matter levels with aerodynamic diameter <10μm (PM10) and increased morbidity and mortality at various geographical urban areas (1,2). In the present study we have used as a model system the macrophage cell line RAW 264.7 and we sought to compare the induction of proinflammatory mediators in the cell-line by Rofa, Carbon Black, fine fraction and coarse fraction of an air sample collected in the city of Rome, during the winter season. We have evaluated cytotoxicity, arachidonic acid (AA) release, and the production of Tumor Necrosis Factor Alpha (TNF-α) induced by the same concentration of the different particles. By comparing the production of proinflammatory mediators induced by all the kinds of particles, we should elucidate the importance of particle core versus the organic compounds and transition metals adsorbed on urban particles.

Materials and methods

The airborne particulate was collected continuously for 15 days, in Rome, Italy, during February 2001. The sampling site was located in the central urban area characterised by moderate or heavy traffic. In winter season the principal source of the atmospheric pollution in this area are the vehicular traffic and heating systems.
The single particles constituting the coarse and fine fractions were characterised by Scanning Electron Microscopy (SEM), equipped with a system for X-ray microanalysis of the elemental determination by energy dispersion spectrometry and the data were analyzed by cluster analysis methods.
The mouse monocyte/macrophage cell line RAW 264.7 was maintained in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 10% heat-inactivated fetal bovine serum, 2 mM of Glutamax I (complete RPMI 1640 medium). All experiments were performed using RPMI with 1% heat inactivated fetal bovine serum. RAW 264.7 cells were plated at a density of 1.3 x 10^5 cells/well in 96 wells plates. Cell cultures were allowed to adhere overnight, and stimulated with the particles the day after.

Results

The cluster analysis method allowed us to identify in the particulate matter seven groups (clusters) of similar particles in both the coarse and the fine fractions: C-rich particles, Ca-carbonates, Ca-sulphates, silica, silicates, Fe-rich particles and metals (figure 1a,b).
The most significant source of the carbonaceous particulate, C-rich particles,in the urban area of Rome consists of the motor vehicle exhausts. C-rich particles were more abundant in the fine fraction (71.5%) than in the coarse fraction (29.4%).

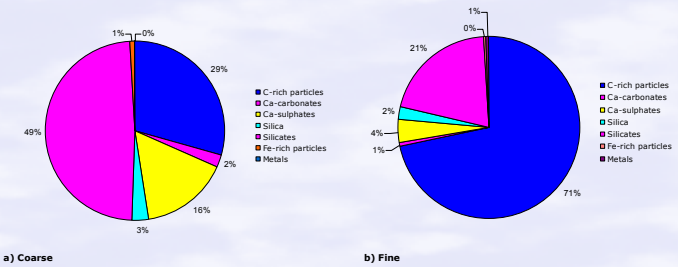

Fig.1. Principal clusters identified by Cluster Analysis in **a)** coarse fraction and in **b)** fine fraction

AA release after 5 h of cell treatment (figure 2) showed that fine fraction induced at 30 μg/ml and 120 μg/ml a significant ($p<0.05$) release of 199 ± 39% and of 208 ± 25.8% respectively. Coarse fraction–induced AA release was not significant. Rofa-induced of AA was lower than the level of both urban fractions, whereas CB had no effect on AA release. LPS at a concentration of 1 μg/ml significantly ($p<0.001$) induced a release of [^{3}H]AA of 280 ± 35.7 % (n=6).

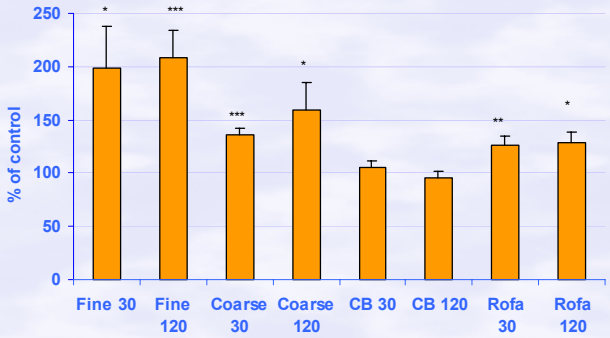

Fig. 2. Effect of Carbon Black, Rofa, Fine and Coarse urban particles on [^{3}H]AA release in RAW 264.7 cells. Cells were prelabelled with [^{3}H]AA and then incubated for 5 h with the particles (30 μg/ml and 120 μg/ml). The radioactivity released by untreated cells was taken as 100%. Values are means ± S.E.M. of six independent experiments assayed in triplicate. Values with asterisk are significantly different from corresponding control cells.

As shown in figure 3, both urban fractions dose-dependently increased TNF-a production at both time point. After 24 h of treatment TNF-α production induced by coarse particles at 30 μg/ml decreased significantly ($p<0.01$). At 120μg/ml the decrease was not significant from 2404 ± 377 pg/ml after 5 h to 1502 ± 263 pg/ml after 24 h.
Fine fraction markedly induced a TNF-α release ($p<0.005$) at 30 μg/ml and ($p< 0.05$) at 120 μg/ml after 5 h of treatment. After 24 h of incubation fine fraction at both concentration did not show any significant decrease of TNF-α production. Carbon black and Rofa both had a lower effect on TNFα release and decreased significantly ($p<0.005$) after 24 h of incubation.

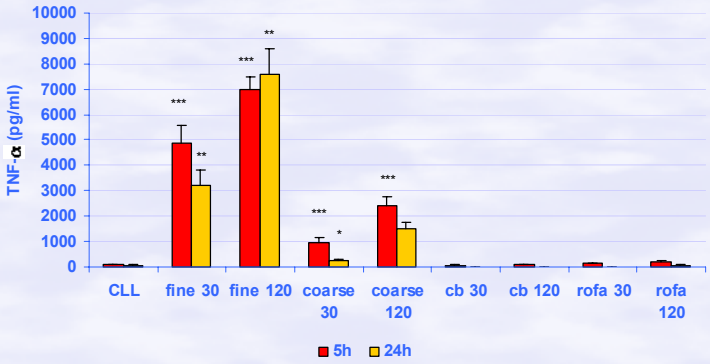

Fig. 3. Effect of Carbon Black, Rofa, Fine and Coarse urban particles on TNF-α production in RAW 264.7 cells.
The cells were incubated in the presence of the particles A: 30 μg/ml and B: 120 μg/ml for 5 h and 24 h.
Values are means ±S.E.M. of six independent experiments assayed in duplicate.
Values with asterisk are significantly different from corresponding control cells.

Conclusions

In conclusion, our data indicate that fine urban particles collected during the winter season in the city of Rome induced in vitro an inflammatory reaction more than the coarse urban particles. The use of carbon black and Rofa, with granulometric size within the range of fine particles evidentiated an inflammatory reaction less marked than that induced by the fine urban fraction, indicating that organic compounds adsorbed on the particles surface are responsible for cytokines and inflammatory mediators production.

1) Katsouyanni, K., Touloumi, G., Samoli, E., Gryparis, A., Le Tertre, A., Monopolis, Y., Rossi, G., Zmirou,D., Ballester, F., Boumghar, A., Anderson, H.R., Wojtyniak, B., Paldy, A., Braunstein, R., Pekkanen, J., Schindler, C. and Schwartz J. 2001. Epidemiology 12, 521-531.

2) Zanobetti, A., Schwartz, J., Dockery, D.W. 2000. Environ. Health Perspect. 108, 1071-77.