OXIDATIVE POTENTIAL OF ENVIRONMENTAL PARTICULATE MATTER FROM SITES WITH VARYING TRAFFIC DENSITY: CORRELATION BETWEEN ANTIOXIDANTS DEPLETION AND ARACHIDONIC ACID RELEASE

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AIRNET
A Thematic Network on Air Pollution and Health

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INTRODUCTION and STUDY DESIGN

World-wide, epidemiological studies have consistently demonstrated an association between airborne concentrations of particulate matter (PM) and cardiovascular and pulmonary morbidity and mortality. The specific contribution of traffic PM to this effect is largely unknown. A European Union funded project (Health effects of particles from motor engine exhaust and ambient air pollution; HEPMEAP) was established to assess the toxicological potential of PM collected from various sites across Europe with established contrasts in traffic density. The project sought to test the hypothesis that the toxicity of ambient PM samples was related to their chemical composition. Thirty-two coarse (PM2.5-10) and fine (PM0.1-2.5) samples were collected from a variety of European metropolitan and rural areas using a high volume impactor and their oxidative activity assessed in a synthetic model of the respiratory tract lining (RTLF) fluid by determining their capacity to deplete ascorbate, urate and reduced glutathione (200μM of each) following a 4h incubation (37ºC, pH7.4) at a PM concentration of 50μg/ml.The PM2.5-10 and PM0.1-2.5 inflammatory response was evaluated by measuring the release of arachidonic acid (AA), tumour necrosis factor alpha and interleukin-6 from a monocytic/macrophagic cell line (RAW 264.7) following exposure to 20 and 60mg/cm^2. The AA release induced by the thirty two coarse samples at 60mg/cm^2 in RAW 264.7 cells supernatants was also shown to be strongly associated ($p<0.001$) with the loss of GSH ($r^2=0.61$) and ascorbate ($r^2=0.36$) from the synthetic RTLF. As the loss of these antioxidants is largely driven by the concentration of water leachable transition metals the impact of metal chelation on the macrophage AA response was investigated. Transition metals have been implicated in determining toxicity mainly through their ability to generate reactive oxygen species (ROS) (1). ROS generated through Fenton-like chemistry may activate mitogen-activated protein kinases (MAPK) cell signalling cascades (2), which in turn may phosphorylate and activate cytosolic phospholipase (3). Four samples eliciting the greatest AA release, according to our screening data and collected from four different sites (table 1) were studied. Increased availability of AA may increase prostaglandins and leukotrienes synthesis which may play a role in the changes in airway tone associated with the allergic response. In order to evaluate the role of PM iron content in inducing AA release samples were pre-treated with the membrane-impermeable iron-cupric metal chelator diethylenetriaminepentaacetic acid (DTPA). This chelator does not cause any cytotoxicity at the concentrations of 0.1 mM and 1mM used in the present study. Ferrous ion content was determined using the chromogenic chelator bathophenantroline disulphonate which forms a ferrous complex that absorbs strongly at 535nm. Total iron was determined after a pre-incubation of PM with ascorbate. Coarse particles at 60 μg/cm^2 but not at 20 μg/cm^2 showed a marked AA release which was significantly reduced with DTPA pre-treatment as shown in Fig.1. The effect of DTPA on fine PM-induced AA release was less clear. DTPA also induced a significant reduction in AA release induced by iron-rich residual oil fly ash (ROFA) particles suggesting a clear involvement of iron in the induction of A2 phospholipases in this model.

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2) Imrich A., Ning Y.Y., and Kobzik L. (2000) Toxicol. Appl. Pharmacol. 167: 140-150.
3) Qiu Z-H., Gijon A., de Carvalho M.S., Spencer D.M., and Leslie C.C. (1998) J. Biol.. Chem. 273: 8203-8211.

RESULTS

Table 1. Iron Content

Samples Codes	Location	Time of the year	Iron Content μM/mg PM
10 & 13 (NL)	Sassenheim	December	19.1 +/- 0.89
18 & 19 (NL)	Amsterdam	March	58.8 +/- 0.89
24 & 25 (DL)	Ostbanhof	April	190.2 +/- 4.73
HIA (NL)	Dutch Tunnel		102.2 +/- 3.22

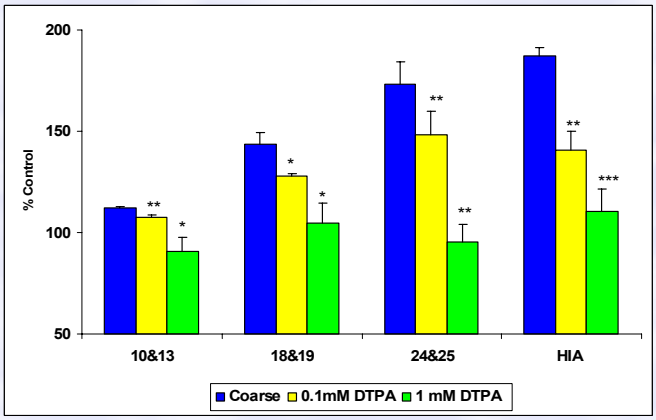

Fig. 1. Effect of coarse particles on [^{3}H]AA release in RAW 264.7 cells. Cells were pre-labelled with [^{3}H]AA and then incubated for 5h with the particles at 60 μg/cm^2 with and without DTPA. The radioactivity released by untreated cells was taken as 100%. Values are means of three experiments assayed in triplicate. The iron-chelator DTPA significantly (*p<0.05; **p<0.01; ***p<0.005) inhibited the particles-induced AA release.

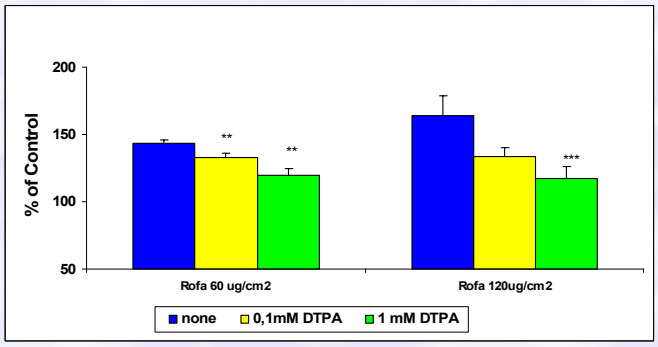

Fig. 2. Effect of DTPA on Rofa-induced [^{3}H]AA release in RAW 264.7 cells. Cells were prelabelled with [^{3}H]AA and then incubated for 5h with Rofa particles at 60 and 120 μg/cm^2 with and without DTPA. The radioactivity released by untreated cells was taken as 100%. Values are means of six experiments assayed in triplicate. DTPA significantly (** p <0.01; ***p<0.005) inhibited the Rofa-induced AA release.

Conclusions

These data indicate that (a) the oxidative potential of PM is strongly related to their capacity in induce AA release from macrophages and (b) that this relationship can be explained in terms of the bioavailable pool of iron in these PM samples. These relationships were only apparent in the coarse fraction. The absence of a clear cut association with PM0.1-2.5 may reflect differences in cellular uptake into the macrophages compared with coarse PM.