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Original Contribution

Oxidative stress and lipid mediators induced in alveolar macrophages by ultrafine particles

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Abstract

In ambient aerosols, ultrafine particles (UFP) and their agglomerates are considered to be major factors contributing to adverse health effects. Reactivity of agglomerated UFP of elemental carbon (EC), Printex 90, Printex G, and diesel exhaust particles (DEP) was evaluated by the capacity of particles to oxidize methionine in a cell-free in vitro system for determination of their innate oxidative potential and by alveolar macrophages (AMs) to determine production of arachidonic acid (AA), including formation of prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4), reactive oxygen species (ROS), and oxidative stress marker 8-isoprostane. EC exhibiting high oxidative potential induced generation of AA, PGE_2, LTB_4, and 8-isoprostane in canine and human AMs. Printex 90, Printex G, and DEP, showing low oxidative capacity, still induced formation of AA and PGE_2, but not that of LTB_4 or 8-isoprostane. Aging of EC lowered oxidative potential while still inducing production of AA and PGE_2 but not that of LTB_4 and 8-isoprostane. Cellular ROS production was stimulated by all particles independent of oxidative potential. Particle-induced formation of AA metabolites and ROS was dependent on mitogen-activated protein kinase kinase 1 activation of cytosolic phospholipase A_2 ($cPLA_2$) as shown by inhibitor studies. In conclusion, $cPLA_2$, PGE_2, and ROS formation was activated by all particle types, whereas LTB_4 production and 8-isoprostane were strongly dependent on particles' oxidative potential. Physical and chemical parameters of particle surface correlated with oxidative potential and stimulation of AM PGE_2 and 8-isoprostane production.

Keywords: Ultrafine particles; Oxidative stress; Phospholipase A_2; Lipid mediators; Prostaglandin E_2; Leukotriene B_4; 8-Isoprostane; Alveolar macrophages; Free radicals

Introduction

Worldwide, acute exposure to inhaled ambient particles has been found to be associated with adverse health effects. The fraction of ultrafine particles in the ambient aerosol is considered a major factor contributing to adverse health effects, including pulmonary and cardiovascular diseases [1–4]. The size distribution of particles at urban sites indicates that the ultrafine fraction (<0.1 μm) represents about 72% of the number concentration of particles in the ambient aerosol, whereas their mass concentration is negligible (1.1%)

Abbreviations: AMs, alveolar macrophages; EC, elemental carbon; DEP, diesel exhaust particles; ESR, electron spin resonance; LTB_4, leukotriene B_4; PGE_2, prostaglandin E_2; TXB_2, thromboxane B_2; $cPLA_2$, cytosolic phospholipase A_2; $iPLA_2$, Ca^{2+}-independent phospholipase A_2; $sPLA_2$, secretory phospholipase A_2; COX, cyclooxygenase; 5-LO, 5-lipoxygenase; CL, chemiluminescence; BEL, bromoenol lactone; MKK1, mitogen-activated protein kinase kinase 1; p38 MAPK, p38 mitogen-activated protein kinase; ERK 1,2, extracellular signal-regulated kinase 1,2.

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compared to the fine particle fraction (<2.5 μm) [5]. Due to their very small diameters (<0.1 μm), the ultrafine particles are predominantly deposited in the periphery of the lungs and interact with cells of the alveolar region such as alveolar macrophages (AMs) and epithelial type I and II cells. The biologic reactivity of ultrafine particles is supposed to be determined by their large specific surface area. Our previous study with AMs indicated that the effect of ultrafine particles of elemental carbon (EC) and of titanium dioxide (TiO_2) on lipid mediator generation is determined by the specific surface area rather than the mass concentration of the particles [6]. Oberdörster et al. [7] have shown that instillation of ultrafine particles of TiO_2 (diameter 21 nm, surface area 50 m^2/g) into the lungs of rats elicited a stronger inflammatory response than that of fine TiO_2 particles (diameter 250 nm, surface area 6.5 m^2/g). These studies reveal that the toxicity of the particles is related to their surface area. Findings of Brown et al. [8] confirmed that increased numbers of neutrophils in the bronchoalveolar lavage fluid induced by instilled ultrafine and fine polystyrene particles in rat lungs correlated with the surface area of these particles. Furthermore, there is evidence that ultrafine particles elicit oxidative stress [8,9]. However, the molecular mechanisms underlying these particle-induced effects are still not well known.

A recent study has shown that AMs obtained from children contained ultrafine carbonaceous particles singly but also as aggregates. The percentage of particle-containing AMs was higher in children whose parents lived on a main road compared to those living in a quiet residential area [10]. Furthermore, ultrafine particles have also been found in AMs from healthy nonsmoking adults, suggesting an environmental exposure to ultrafine particles [11]. These observations indicate that AMs are relevant target cells for ultrafine particles.

For the present in vitro study we hypothesized that the oxidative potential of ultrafine particles reflects their surface reactivity, which is decisive for their biologic impact. We therefore analyzed the oxidative potential of various ultrafine carbon particles in a cell-free in vitro system as a measure for their surface reactivity. In addition, we evaluated in primary canine or human AMs their biologic response to ultrafine particles especially focusing on lipid mediator synthesis and lipid peroxidation indicating oxidative stress. AMs as part of the primary pulmonary defense system produce pro- and anti-inflammatory mediators, including metabolites of arachidonic acid such as leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2), after interference with particulate matter [6]. As models for ultrafine particles we used agglomerates of ultrafine particles of EC, Printex 90, Printex G, and diesel exhaust particles (DEP). EC consists of spherical primary particles with a uniform size of a few nanometers (5–10 nm), which combine to randomly form agglomerates and simulate the carbonaceous matrix of diesel exhaust particles [12,13]. Printex 90 and Printex G are both ultrafine carbonaceous particles with different specific surface areas. They are commercially available and used as carbon black particles for various technical applications. DEP (Standard Reference Material 1650a) represents ultrafine environmental particles [12].

Materials and methods

Materials

Phosphate-buffered saline (PBS) with or without Ca^{2+} and Mg^{2+} was purchased from Biochrome (Berlin, Germany); RPMI was from PAA Laboratories (Linz, Austria); fetal calf serum, penicillin, streptomycin, and amphotericin were from Life Technologies (Eggenstein, Germany); ^{14}C-labeled arachidonic acid was from Amersham Pharmacia Biotech (Freiburg, Germany); arachidonyl trifluoromethyl ketone ($AACOCF_3$), diphenyleneiodonium chloride, PD 98059 (2′-amino-3′-methoxyflavone), PD 184352 (2-(2-chloro-4-iodophenylamino)-*N*-cyclopropylmethoxy-3,4-difluorobenzamide), and SB 203580 (4-(4-fluoro-phenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole) were from Calbiochem (Bad Soden, Germany); bromoenol lactone (BEL) and thioetheramide-PC (1-palmitylthio-2-palmitoylamido-1,2-dideoxy-*sn*-glycero-3-phosphorylcholine) were from Cayman (Ann Arbor, MI, USA); lucigenin and zymosan were from Sigma (Deisenhofen, Germany); methionine, methionine sulfoxide, and all other chemicals (analytical or high-performance chromatography grade) were from Merck (Darmstadt, Germany).

Particle characteristics

Ultrafine particles of EC were generated by spark discharging according to Roth et al. [13]. The EC particles consisted of individual particles with a diameter of 5–10 nm and had a BET specific surface area of 750 ± 150 m^2/g (n = 50) [13]. BET (Brunauer–Emmett–Teller surface area) is a well-known method to determine the total surface area of a material by adsorption of liquid nitrogen [13]. Ultrafine Printex 90 particles had a diameter of 14 nm and a specific surface area of 300 m^2/g, whereas ultrafine Printex G particles had a diameter of 51 nm and a specific surface area of 30 m^2/g. Both types of Printex particles were purchased from Degussa (Frankfurt, Germany). Diesel exhaust particles (Standard Reference Material 1650a) were purchased from NIST (Washington, DC, USA) and consisted of individual particles with a diameter of 20–40 nm as observed by electron microscopy and had a specific surface area of 108 m^2/g.

Ultrafine particles of EC, Printex 90, Printex G, and diesel exhaust were suspended in distilled water by repeated vortexing (5 times for 3 s) and sonification (1 min). Because ultrafine particles formed agglomerates in suspension despite vigorous mixing, the cells were