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Effects of Atmospheric Particles and Several Model Particles of Particulate Matter Components on Human Monocyte-Derived Macrophage Oxidative Responses

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Abstract

Atmospheric particles are ingested by alveolar macrophages in the lung. The macrophages release reactive oxygen species on ingesting the particles. The aim of this study was to investigate the effect of particulate matter components on the release of reactive oxygen species from macrophages. We compared the effect of particulate matter with that of several model particles of particulate matter components, such as diesel exhaust particles, silica particles, two kinds of carbon black particles (a laser printer toner and an aggregation of laser printer toner), and 3-nitrobenzanthrone-coated carbon black particles, on the time course of induction of lucigenin-dependent chemiluminescence in human monocyte-derived macrophages. The particulate matter strongly induced chemiluminescence at lower concentrations. We observed similar chemiluminescence responses for particulate matter and 3-nitrobenzanthrone-coated carbon black particles. This result suggests that materials such as 3-nitrobenzanthrone are important contributors to the induction of reactive oxygen species by particulate matter. We also examined hydroxyl radical generation by the 2-deoxy-D-ribose method. Hydroxyl radical generation by particulate matter and diesel exhaust particles was greater than that by the other samples (the Mann–Whitney U test: $p < 0.05$). These results suggest that diesel exhaust and 3-nitrobenzanthrone-coated carbon black particles are insufficient as model particles of particulate matter components.

Keywords: Particulate matter; Diesel exhaust particles; 3-Nitrobenzanthrone; Superoxide; Hydroxyl radical; Macrophage

Abbreviations: PM: Particulate Matter; ROS: Reactive Oxygen Species; CL: Chemiluminescence; SP: Silica Particles; DEP: Diesel Exhaust Particles; 3-NBA: 3-Nitrobenzanthrone

Introduction

Fine particulate air pollution is associated with cardiopulmonary and lung cancer mortality [1-4]. Particulate matter (PM) is a complex mixture of particles from different sources. PM is known to include various components such as silica particles (SP), diesel exhaust particles (DEP), and carbon particles; however, the relationship between chemical composition and toxic effects of PM inhalation is not clear.

Evidence that the source and composition of PM are important determinants of its health effects has been derived from studies that demonstrated increased respiratory symptoms in people residing in close proximity to busy roads. Exposure to traffic-related pollution has been associated with retarded lung development [5] and enhanced allergic and respiratory symptoms in children attending schools [6]. PM has been compared with DEP for its biological effects. PM induces the release of pro-inflammatory cytokine [7] and reactive oxygen species (ROS) from macrophages [8-9].

Apart from acting as the primary nonspecific defense of the lung against inhaled PM, alveolar macrophages modulate the inflammatory response by secreting various mediators [10-13]. Oxidative stress may underlie the adverse health effects of many inhaled pollutants [14,15].

We sampled PM for *in vitro* experiments using a high-volume air sampler with an Omnipore membrane filter. We previously showed that PM induced lucigenin-dependent chemiluminescence (CL) in human monocyte-derived macrophages at low to trace concentrations but inhibited this response at high concentrations, and that DEP completely nullified the CL response [9]. Kleinman et al. showed that concentration-dependent decreases in superoxide production from alveolar macrophages of rats with ambient particles [8]. The superoxide response was induced at low to trace concentrations but reduced at high concentrations of PM may be an important property for the evaluation of the relationship between the chemical composition and biological effects of PM.

The aim of this study was to investigate the effect of PM components on the release of ROS from macrophages. We compared the effect of PM with that of several model particles of PM components, such as DEP, SP, carbon black particles, and 3-nitrobenzanthrone (3-NBA)-

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coated carbon black particles (MCC) on the release of ROS from human monocyte-derived macrophages. 3-NBA, which is found in DEP and airborne PM is a polycyclic aromatic hydrocarbon and powerful mutagen [16,17].

Material and Methods

Particle samples

PM: The method of PM collection has been described [9]. In brief, approximately 10 mg of PM was collected using a high-volume air sampler (HV-500; Sibata Scientific Technology LTD., Souka, Japan) with an Omnipore membrane filter (JMWP14225, poly tetrafluoroethylene, filter type 5.0 μm JM; Japan Millipore, Tokyo, Japan) on the roof of a building for approximately 6 h. PM was washed from the filter using a vortex mixer with 10 mL of water and a magnetic stir bar (size 35 × φ16 mm) in a 50 mL centrifuge tube for 3 min [18]. On a dry weight basis, approximately 70% of PM on the filter was recovered as the PM sample.

DEP: DEP samples were kindly provided by Dr. Kobayashi of the Japanese National Institute for Environmental Studies (Tsukuba, Japan).

Two carbon black particle samples: Nano-particles from a laser printer toner (NC: Mitsubishi carbon black # 3950; Mitsubishi Chemical Corporation, Tokyo, Japan) and aggregations of NC particles (FC) were prepared. The FC sample was prepared as follows: ethanol (20 mL) and tri-n-octanoin (0.5 mL) were added to 1 g of NC and shaken vigorously at 60°C. The samples were then dried by heating at 60 - 80°C for several hours and 45°C overnight.

MCC: Ethanol (20 mL) and tri-n-octanoin (0.5 mL) with 3-NBA (100mg) were added to 1 g of NC and shaken vigorously at 60°C. The samples were then dried by heating at 60 - 80°C for several hours and 45°C overnight.

SP: Silica microspheres (Polysciences Inc., Warrington, PA, USA) with particle diameter 50 ± 10 nm were used.

Sample preparation

Each sample was dried and heat sterilized at 80°C for 24 h. They were then ultrasonicated (I-A-4201; Kaijo Denki Co., Hamura, Japan) in sterilized Hanks' balanced salt solution (HBSS) at 10mg/mL.

Each sample was suspended in excess concentration of fetal bovine serum (FBS) (Gibco; Invitrogen Co., Carlsbad, CA, USA), incubated for 15 min at 37°C, and spin-washed three times in HBSS at 900 g for 20 min. Pellets were resuspended at stepped suspension concentrations from 10/13 mg to 10 mg/mL. The suspensions were stored at 4°C until further use.

Particle size distribution

The particle size distribution of each sample was measured using an electrophoretic light scattering spectrophotometer (ELS-8000HW; Otsuka Electronics Co., Ltd., Hirakata, Japan) with appropriate dilutions from 0.1 % to 0.001% in Milli-Q water.

Cell isolation

The method of cell isolation has been described [9,19]. In brief, heparinized blood was obtained from healthy donors by veni-puncture and diluted 1:1 in HBSS. Monocyte-lymphocyte fractions were isolated by Ficoll density centrifugation and plated them in 9-cm-diameter plastic tissue culture dishes for monocyte adherence [20]. The adhering cells were cultured for 8 days in HEPES modified RPMI1640 medium (Sigma Chemical Co., St.Louis, MO, USA) with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. This culture medium was changed every 2 days. Adherent cells were separated by pipetting with serum free-HBSS after 6 days, and suspended in serum free-RPMI1640 at 4°C. This study was approved by the ethical committee of Osaka Prefectural Institute of Public Health institutional review board, human subjects protocol 0210-24.

Cell viability assay

Cell viability was determined by the MTT assay [21]. In brief, cells were seeded into 96-well plates, with each well containing 1×10^4 cells. Prior to the cell viability assay, the cells were incubated with the various particle samples and positive control of 5 % sodium dodecyl sulfate for 3 h at 37°C, using four wells for each concentration. The medium was aspirated, and 20 μL of fresh MTT (5g/L PBS) was added to each well. After overnight incubation, 100 μL each of isopropanol, TritonX and hydrochloric acid was added to each well and vibrated to dissolve the depositor. After centrifugation, the absorbance of the supernatant from each well of the 96-well plate was measured using an enzyme-linked immunosorbent assay plate reader at 550-690 nm. Cell viability was assessed by the ratio of absorbance of the NS-398-treated cells to that of the controls.

CL measurements

The method of CL measurement for 6-day-old human monocyte-derived macrophages exposed to various mineral fibers has been described [9,19]. In brief, isolated cells (1×10^5 cells) were transferred into a luminometer tube containing 65 μL of particle sample suspension, 10% FBS, and 0.1 mM lucigenin. The final volume of each tube was 1 mL. The light emission for each sample was recorded for 180 minutes at 15-min intervals using a luminescence reader (BLR-201; Aloka Co., Mitaka, Japan).

Hydroxyl radicals assay

Hydroxyl radicals were determined by the 2-deoxy-D-ribose method [22]. In brief, 2-deoxy-D-ribose (4 mM), suspended cells (1×10^5 cells), and a PM sample (350 μg) were mixed and incubated at 37°C for 1 h. A solution of 5 mM 2-thiobarbituric acid and 1% trichloroacetic acid (250 μL) in water was added to the supernatant (750 μg) of the centrifuged mixture in a glass test tube with a lid. The tube was boiled for 30 min and cooled, and the absorbance of the supernatant was measured at 532 nm. The molar absorption coefficient was 1.5×10^5.

Statistical analysis

Differences between the two experimental groups were examined for statistical significance using the Mann–Whitney U test. Differences with $p < 0.05$ were considered significant.

Results

Particle size distribution

The results for each sample are presented in Figure 1. The average particle size and standard deviation (nm) for each sample: PM, 74.7 ± 24.6; DEP, 82.5 ± 89.1; SP, 72.9 ± 15.7; MCC, 238.8 ± 135.6; FC, 132.8 ± 38.0; and NC, 1.8 ± 0.0. The particle size distributions for MCC and DEP did not show continuous transitions, and MCC contained more large particles than the other samples.

Cell viability

Figure 2 shows the cell viability of each sample as determined

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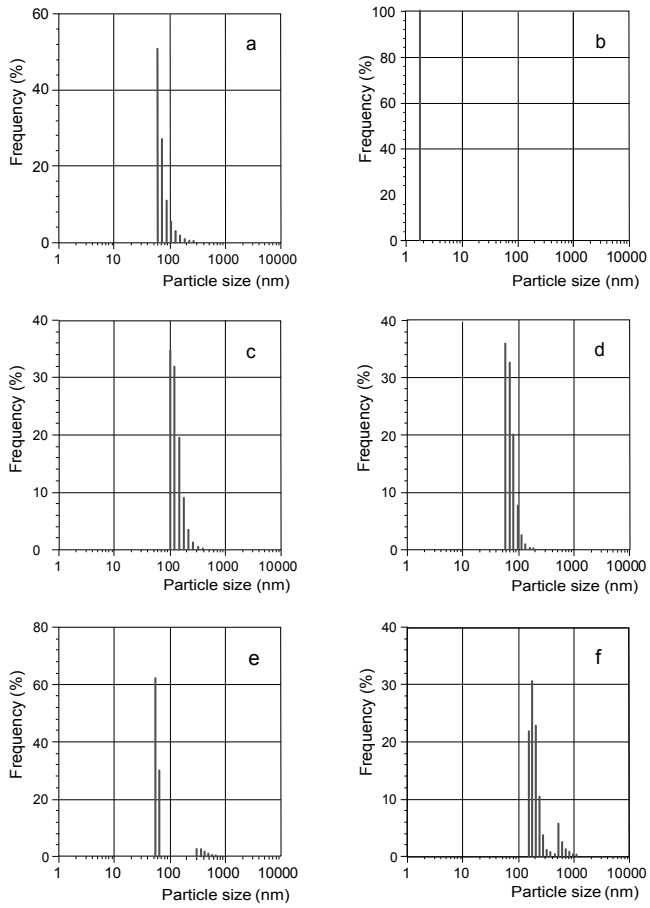

Figure 1: Particle size distributions with an electrophoretic light scattering spectrophotometer. The vertical axis displays frequency (%). The horizontal axis displays particle size on a logarithmic scale (nm). The average size and standard deviations (nm) were: (a) PM, 74.7 ± 24.6; (b) NC, 1.8 ± 0.0; (c) FC, 132.8 ± 38.0; (d) SP, 72.9 ± 15.7; (e) DEP, 82.5 ± 89.1; and (f) MCC, 238.8 ± 135.6.

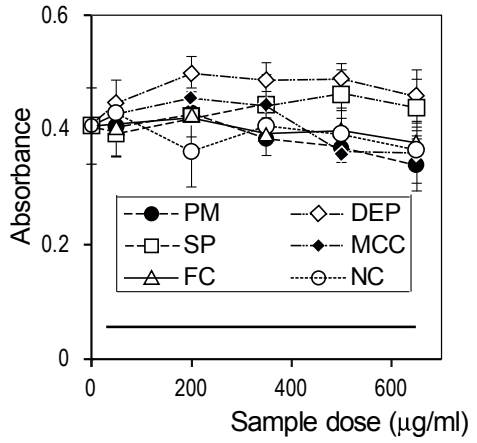

Figure 2: Cell viability for each sample as determined by the MTT assay. The vertical axis displays absorbance. The horizontal axis displays sample concentration (μg/mL). The line indicates a lever of positive control absorbance (5% sodium dodecyl sulfate). *$p < 0.05$ by the comparison with the controls. Statistical analysis by the Mann–Whitney U test (n = 4).

by the MTT assay. Human monocyte-derived macrophages treated with 50, 200, 350, 500, and 650μg/ml of each sample for 3 h showed no significant decrease compared with the controls ($P > 0.05$); this indicates that the samples had no significant effect on cell viability (MTT accumulation) at the experimental concentrations.

CL responses

Figure 3 demonstrates the time course of the CL response of human monocyte-derived macrophages exposed to each sample at concentrations of 50, 200, 350, 500, and 650 μg/ml. A CL peak appeared at 45-min in the controls warmed to 37°C at the start of the measurements. The results for NC and FC were similar to those for the controls at all concentrations. Increased CL response was observed for PM, SP, and MCC. However, concentration-dependency was observed only for SP. The CL peak at 45-min disappeared for PM but decreased for MCC. The highest CL values for PM and MCC were observed at 200 μg/mL, and the highest CL values for all samples were observed at 200 μg/mL PM. Decreases in the CL response were observed at all DEP concentrations.

Hydroxyl radical response

Figure 4 shows hydroxyl radical generation by the 2-deoxy-D-ribose method. Human monocyte-derived macrophages were treated with 350 μg/mL of each sample for 1 h. Hydroxyl radical generation was not detected in the containing only macrophages. A statistically

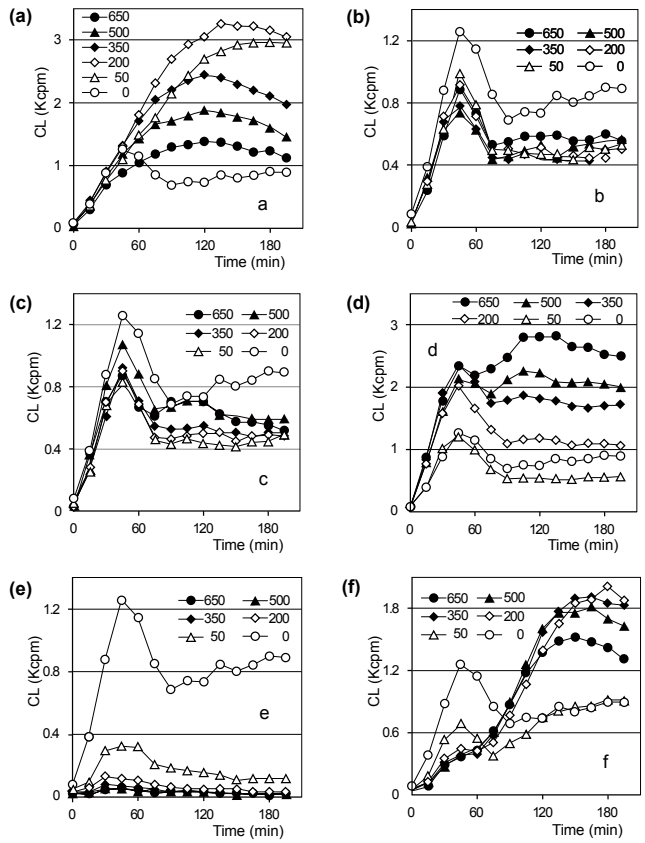

Figure 3: Time course of the CL response in macrophages for each sample. The vertical axis displays CL (Kcpm). The horizontal axis displays time (min). Each symbol in the legend denotes concentration (μg/mL) (n = 3). (a) PM, (b) NC, (c) FC, (d) SP, (e) DEP, and (f) MCC.

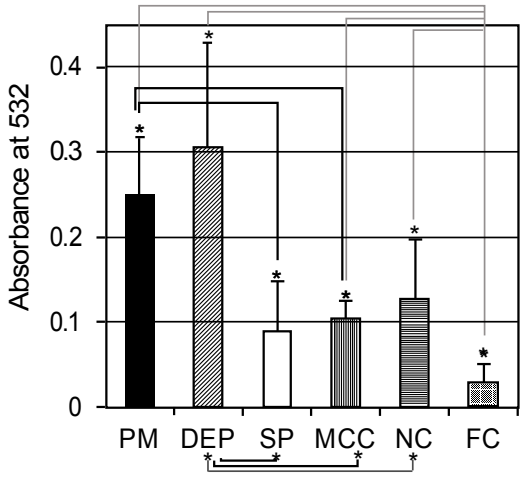

Figure 4: **Hydroxyl radical generation by 2-deoxy-D-ribose method.** The vertical axis displays absorbance. *p < 0.05 by the comparison with two groups. Statistical analysis by the Mann–Whitney U test (n = 6).

significant difference (P < 0.05) by the Mann–Whitney U test was observed among: FC and PM; FC and DEP; FC and MCC; FC and NC; DEP and SP; DEP and MCC; DEP and NC; PM and SP; and PM and MCC. Hydroxyl radical generation by PM and DEP was greater than that by the other samples.

Discussion

This study demonstrated that PM and MCC exerted scavenging and enhancing effects on lucigenin-dependent CL in macrophages, that DEP exerted only scavenging effects on CL. In addition, SP showed s comparatively stable dose-response relationship. No CL response could be observed for NC and FC, suggesting that carbon black particles did not induce superoxide release by macrophages. PM may include various components such as carbon particles, DEP, SP, and etc. SP can induce silicosis and the release of ROS from ingesting macrophages. However, the peak CL value at the 50 μg PM was the highest of all CL values at 50 μg because of SP. Our results suggested that low PM concentrations have a greater ability to induce superoxide release from ingesting macrophages than low SP concentrations. We also found that the CL response due to PM was similar to that due to MCC. The similarities between PM and MCC were that their low concentratuons (200 μg/mL) induced the highest CL response and that their CL responses at 45 min were scavenged. These results suggested that 3-NBA at low concentrations enhances the ability to induce superoxide release from ingesting macrophages.

The suppression of CL response was not due to decreased cell viability as evidenced by the lack of cytotoxicity in the cells exposed to MCC, PM, and DEP. Although the mechanism for the suppression of CL response is unknown, the component common to PM, MCC, and DEP is 3-NBA. The amount of 3-NBA in the MCC sample was greater than that in the PM samples. The CL response for MCC was lower than that for PM, and the rate of CL increase from 75 to 105 min for MCC was higher than that for PM. We propose that MCC exerts stronger effects than PM on the scavenging and enhancing of the superoxide response.

Kleinman et al. observed a concentration-dependent decrease in production of superoxide radicals by macrophages in response to ambient particles collected at sites in The Netherlands with high vehicular traffic [8]. We propose that the induction of high CL responses by low PM concentrations is an important indicator of the toxic properties of inhaled PM. Li et al. reviewed the role of oxidative stress in asthma emphasizing on the pro-oxidative effects of DEP and associated chemicals such as polycyclic aromatic hydrocarbons [23]. The polycyclic aromatic hydrocarbon 3-NBA is a particularly powerful mutagen. 3-NBA has a ketone and a nitro group and forms a DNA adduct at the nitro group position. However, it is not a quinone. The MCC result suggests that a single component could have inductive and inhibitory effects on the superoxide response of ingesting macrophages, and that polycyclic aromatic hydrocarbons may be important contributors to the oxidative effects of PM.

DEP is a standard sample collected at a facility of the Japanese National Institute for Environmental Studies and contains many components. Although the amount of 3-NBA in the DEP sample was less than that in the MCC sample, the DEP sample included various other components, some of which could be common to DEP and PM. Sawyer et al. indicated that urban dust and DEP suppressed superoxide release by normal human alveolar macrophages in response to phorbol-12,13-myristate acetate (PMA) stimulation [24]. Aam and Fonnum observed that the organic extract of DEP decreased the amount of ROS released by rat alveolar macrophages stimulated by PMA [25]. We could observe only a scavenging effect on the CL response for DEP, probably because it resulted from several components of DEP. Our results suggest that DEP is not a suitable model particle of PM because it contains many components not common to PM.

The results of the hydroxyl radical assay suggest that MCC is not a suitable model of PM for the induction of ROS from ingesting macrophages. Hydroxyl radicals are a product of the reaction between a superoxide and hydrogen peroxide, known as the Haber–Weiss reaction [26]. The ferrous iron-catalyzed hydrogen peroxide reaction is known as Fenton's reaction [27]. Therefore, hydroxyl radicals may be induced to the same extent as PM using NC coated with 3-NBA and an iron compound.

In conclusion, our results suggest that the PM effect on the superoxide response of ingesting macrophages is inductive at low to trace concentrations and inhibitory at high concentrations. The experiments with MCC contributed to the understanding of the biological effects of PM. Although MCC does not always show a response similar to that of PM, as shown by the hydroxyl radical assay results, addition of a second component may induce similar reactivity. We suggest that future studies with PM, MCC, and MCC with 3-NBA and additional components will lead to a better understanding of PM compositions that exert biological effects.

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