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Letter

Air purifiers that diffuse reactive oxygen species potentially cause DNA damage in the lung

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ABSTRACT — Several appliance manufacturers have recently released new type air purifiers that can disinfect bacteria, fungi and viruses by diffusing reactive oxygen species (ROS) into the air. In this study, mice were exposed to the outlet air from each of 3 air purifiers from different manufacturers (A, B, C), and the lung was examined for DNA damage, lipid peroxidation and histopathology to confirm the safety of these air purifiers. Neither abnormal behavior during exposure nor gross abnormality at necropsy was observed. No histopathological changes were also observed in the lung. However, significant increase of DNA damage was detected by the comet assay in the lung immediately after the direct exposure for 48 hr to models A and B, and for 16 hr to model B. As for model B, DNA migration was also increased by 2 hr exposure in a 1 m^3 plastic chamber but not by 48 hr exposure in a room (12.6 m^3). Model C did not cause DNA damage. Lipid peroxidation and 8-hydroxy deoxyguanosine (8-OH-dG) was not increased under the conditions DNA damage was detected by the comet assay. The present results revealed that some models of air purifiers that diffuse ROS potentially cause DNA damage in the lung although the mechanism was left unsolved.

Key words: DNA damage, Air purifier, Reactive oxygen species, Air ion, Lung

INTRODUCTION

Air purifiers are commonly used in a house, office, hospital, and so on. Conventional air purifiers only remove particulates such as house dust, pollens, and cigarette smoke by a filter, whereas new type ones have a function to disinfect bacteria, fungi and viruses. For these purposes, some models are equipped with an antibacterial filter or photocatalytic device, and some models diffuse air-ions into the room.

There are several studies on the bactericidal or virucidal effects of air-ions (Mitchell and King, 1994; Fan *et al.*, 2002; Tyagi *et al.*, 2008), and the underlying mechanism is suggested to be degeneration of surface proteins of organisms (Digel *et al.*, 2005). According to the manufacturer's information, active substance of the air purifiers is superoxide or hydroxyl radical, which is a member of reactive oxygen species (ROS). ROS are potentially toxic to living matters. There are few published studies on the safety of these air purifiers although several manufacturers have officially announced on the website that various toxicity tests including genotoxicity test have been conducted by contract research organizations.

In this study, therefore, mice were exposed to the outlet air from these air purifiers and the lung was examined for DNA damage, lipid peroxidation and histopathology to confirm their safety.

MATERIALS AND METHODS

Animals and apparatus

Seven weeks old ICR male mice were purchased from CLEA Japan (Tokyo, Japan), and randomly divided into groups of 5 mice each. They were fed commercial feed (MF, Oriental Yeast, Tokyo, Japan) and tap water throughout the acclimation (1 week) and experimental periods freely. The animal room was kept at 22-24°C with 12 hr light/dark cycle. Animal experiments were conducted according to the guidelines for animal experiments of Iwate University.

Three models of household air purifiers from different manufacturers, A, B and C were tested. According to the manufacturer's information, model A diffuses negative (O_2^-) and positive (H^+) cluster ions, model B diffuses nanoparticles of water including hydroxyl radical, and model C diffuses electrolytic water mist that includes hydroxyl rad-

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ical and hypochlorous acid. Chemicals used without special mention were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Exposure and sampling

For models A and B, exposure was conducted in an air duct connected to their outlet (Fig. 1). Air flow was 0.8 m^3/min for A and 1.0 m^3/min for B at low mode (wind velocity < 0.5 m/sec). For model C, exposure was conducted in an incubator (45 × 46 × 46 cm), because it was not equipped with a fan; and fresh air was introduced into the incubator (0.03 m^3/min) by an air pump during exposure. All of the air purifiers were set to the low mode. The exposures were conducted for 16 hr or 48 hr in respective rooms.

Model B was also tested under two other exposure conditions. (I) The air purifier was put on a side of a small room (12.6 m^3, 2.1 × 2.5 × 2.4 m), and mice were exposed at the opposite side of the room for 48 hr. (II) The air purifier and animal cage were put in an air-tight plastic chamber (1.0 m^3, 0.9 × 1.2 × 0.9 m) to conduct the exposure for 2 hr. Oxygen concentration in the plastic chamber remained practically the same (20.9-20.7%) during exposure.

Immediately after exposure, mice were sacrificed by cervical dislocation to collect the lung for the assessment of its damage.

Assessment of lung damage

DNA damage was assessed by the *in vivo* comet assay and 8-hydroxy deoxyguanosine (8-OH-dG). The comet assay was conducted according to Tsuda *et al.* (2000) and Hashimoto *et al.* (2007), where 50 nuclei/tissue were measured for DNA migration and the mean migration was regarded as the individual level of DNA damage. 8-OH-dG was determined by HPLC equipped with an electrochemical detector. Detailed procedures were described in the next section. Lipid peroxidation was estimated by thiobarbituric acid reactive substances (TBARS) and 8-isoprostane. TBARS were determined according to the method of Kikugawa *et al.* (1992) with a little modification. We omitted the solvent extraction with butanol-pyridine, because transparent samples could be obtained by addition of propanol followed by centrifugation at 3,000 rpm. 8-Isoprostane was determined by an EIA kit (Cayman Chemical, Ann Arbor, MI, USA). Left lobes of the lung were fixed in 10% neutral buffered formalin, routinely processed, and stained with hematoxylin eosin for histopathological examination. Dunnett's test or Student's t test was employed for the statistical analysis, and P value less than 0.05 was considered statistically significant.

8-OH-dG assay

Tissue sample (100-200 mg) was gently homogenized at 0°C with 2 ml of lysing solution (1% Triton X-100, 320 mM saccharose, 5 mM $MgCl_2$, 0.005% BHT, 10 mM Tris, pH 7.5) using a Potter homogenizer. A portion of 1 ml was centrifuged at 600 g for 10 min at 4°C, and the supernatant was carefully discarded. The precipitate was resuspended in 1 ml of the lysing solution and centrifuged under the same conditions. This step was repeated once more. The precipitate was suspended in 0.3 ml of reaction solution (1% SDS, 5 mM Na_2EDTA, 0.005% BHT, 10 mM Tris, pH 8.0) and incubated with 10 µl of proteinase K (17 mg/ml) at 37°C for 60 min. During the incubation, the sample was shaken vigorously every 10 min to facilitate the enzyme reaction.

After the incubation, the sample was centrifuged at 10,000 g for 5 min at 4°C. The supernatant (0.15 ml) was mixed with 0.3 ml of NaI (7.6 M NaI, 20 mM Na_2EDTA, 40 mM Tris, pH 8.0) and 0.6 ml of isopropanol, stirred until filamentous DNA was deposited, and centrifuged at 10,000 g for 5 min at 4°C. The precipitate was resuspended in 1 ml of 70% ethanol and centrifuged under the same

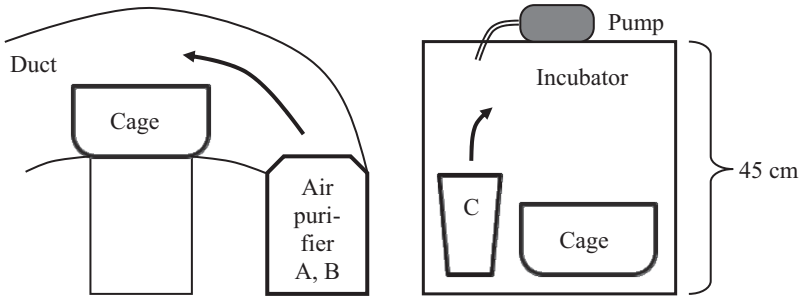

Fig. 1. Schematic diagram of the direct exposure.

conditions. This step was repeated once more. The supernatant was completely discarded to remove ethanol.

The precipitated DNA was dissolved in 0.1 ml of pure water, denatured in boiling water for 3 min, then cooled in ice water. Ten microliters of 200 mM acetic acid buffer (pH 5.3, 1 mM $ZnCl_2$), nuclease P1 (100 U/ml, Yamasa Corporation, Chiba, Japan) and acid phosphatase (100 U/ml) were added to the DNA solution, incubated at 37°C for 30 min, then filtered with a centrifugal filter device (Ultrafree-MC, 0.45 um, Millipore, Bedford, MA, USA) to obtain analytical sample for HPLC. Conditions of HPLC were as follows.

Apparatus : Shiseido Nanospace (Shiseido, Tokyo, Japan)
Column : Inertsil ODS, 3 μm, 3.0 × 50 mm (GL Science, Tokyo, Japan), 35°C
Eluent : 50 mM acetic acid buffer, pH 5.3, 5% methanol, 0.4 ml/min
Detector : UV (275 nm), ECD (Ox 0.6 V)

Ozone measurement

Ozone concentration in the outlet air (model A and B) or in the incubator (model C) was measured by detection tubes (Komyo Rikagaku Kogyo, Kanagawa, Japan).

RESULTS

Neither abnormal behavior during exposure nor gross abnormality at necropsy was observed. No changes were also detected by histopathological examination of the lung directly exposed to model A, B or C for 48 hr (pictures were omitted). The other exposure conditions were not allocated for histopathology.

DNA migration of the lung was significantly increased by 48 hr direct exposure to model A and B, and by 16 hr exposure to model B (Fig. 2). Sixteen hours exposure to model C was not examined, because this model did not increase DNA migration even after 48 hr exposure. As for model B, DNA migration was also increased by 2 hr exposure in a 1 m^3 plastic chamber but not by 48 hr expo-

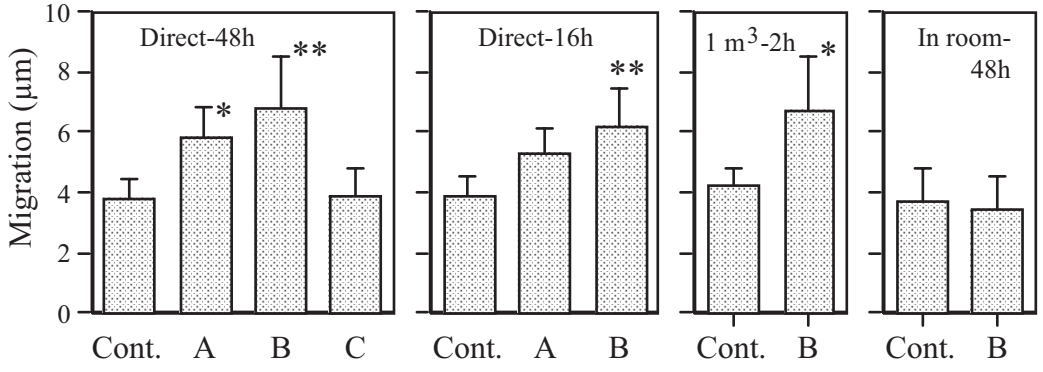

Fig. 2. DNA damage in the lung measured by the comet assay. Mean ± S.D. for 5 mice. *: $p < 0.05$, **: $p < 0.01$.

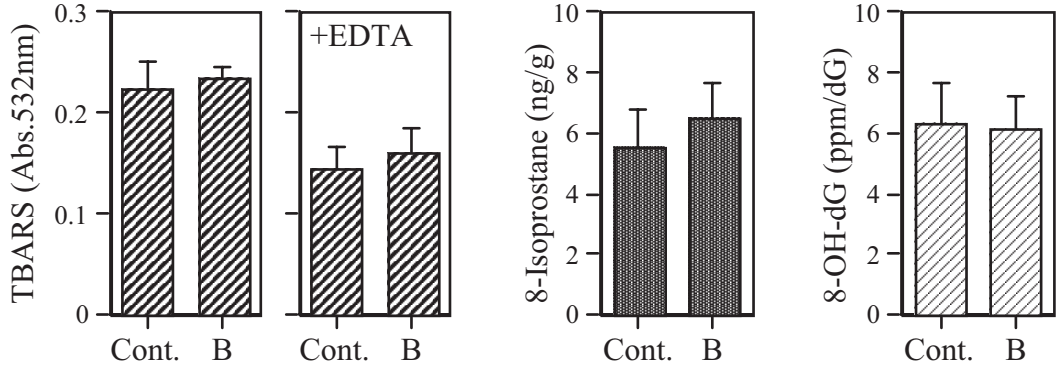

Fig. 3. Lipid peroxidation and 8-OH-dG in the lung of mice exposed directly to model B for 48 hr. Mean ± S.D. for 5 mice.

sure in a small room (Fig. 2).

TBARS, 8-isoprostane and 8-OH-dG were not affected by 48 hr direct exposure to model B (Fig. 3). The other exposure conditions were not examined for these endpoints because above condition caused most severe DNA damage.

Ozone concentration in the exposing air was lower than the detection limit (25 ppb) for all air purifiers tested.

DISCUSSION

According to the instruction manuals, models A and B collaterally generate small amount of ozone. Ozone is a powerful oxidant that causes DNA damage as well as inflammatory reaction in the lung or pulmonary cultured cells (Victorin, 1992; Bornholdt *et al.*, 2002). An *in vivo* study using guinea pig has demonstrated that 72 hr exposure to 1 ppm ozone caused DNA single strand break (SSB) in tracheobronchial epithelial cells, but the same duration at 0.45 ppm did not cause SSB (Ferng, 2002). Occupational exposure limit for ozone recommended by the Japan Society for Occupational Health is 0.1 ppm (JSOH, 2009). In this study, ozone levels in the exposing air were less than 0.025 ppm. Thus, ozone is not involved in the DNA damage caused by air purifiers. It was also demonstrated by histopathological examination that the DNA damage was not attributable to the cellular degeneration or necrosis.

Models A and B diffuse superoxide and hydroxyl radical, respectively, either of which can cause oxidative DNA damage such as modified base and strand break if it reaches the target site. However, they may not react directly with nuclear DNA of pulmonary cells because of their low membrane permeability and/or high reactivity. Thus, inhaled superoxide and hydroxyl radical might have primarily reacted with the epithelial cell lining fluid or cell membrane to cause lipid peroxidation, and indirectly attacked DNA through genotoxic products such as alkylperoxyl radicals, alkoxyl radicals and reactive carbonyls (Burcham, 1998; Blair, 2008). Similar mechanism has been suggested for the development of ozone toxicity (Mehlman and Borek, 1987; Pryor and Church, 1991). However, there was no increase in the levels of TBARS and 8-isoprostane when mice were directly exposed to model B for 48 hr, while the DNA damage was observed. TBA method is a widely used conventional method to evaluate lipid peroxidation in living tissues and foods. 8-Isoprostane is a degradation product of arachidonic acid, and is reported to increase in high-oxygen environment (Vacchiano and Tempel, 1994). The negative results of both TBARS and 8-isoprostane suggest that lipid peroxidation may not be the main cause of the DNA damage. Another possibility may be hydrogen peroxide, a stable and penetrable ROS, which may have been generated before or after inhalation causing oxidative DNA damage of the lung. However, there was no change in the levels of 8-OH-dG, a marker of oxidative DNA damage, under the same conditions as mentioned above.

Model C, which diffuses electrolytic water mist by ultrasonic nebulizer, did not cause DNA damage. However, it is unclear whether the negative result was due to the unique mechanism of this model, because exposure conditions including ROS density were not equalized in this study.

Digel *et al.* (2005) examined bactericidal effects of plasma-generated air ions on several gram-positive strains. They found that the air ions did not damage bacterial DNA but denatured surface proteins. Bacterial cells are enveloped by thick cell wall which consists chiefly of peptidoglycan and teichoic acid. Therefore, the present results do not necessarily contradict their findings and revealed that some models of air purifiers that diffuse ROS potentially cause DNA damage in the lung under strong exposure conditions. However, the mechanism of DNA damage of the lung caused the air purifiers remains to be solved.

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