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Endothelial dysfunction in heart failure rats exposed to real urban air pollution

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Coronary and peripheral endothelial dysfunction has been established in patients with chronic heart failure (HF) [1]. Several recent studies have demonstrated that air pollution (AP) is an environmental health risk factor that is associated with increased cardiovascular morbidity and mortality especially in persons with HF [2–4]. Exposure to ambient AP, including particulate matter (PM), produces systemic inflammation and endothelial damage that develops into cardiovascular diseases [5]. However, these studies were performed under artificial conditions; they used PM samples collected from AP areas or commercially available PM. In order to overcome this limitation, we focused on whether endothelial dysfunction in rats with HF could be induced or exacerbated in response to AP under a real urban ambient environment.

All protocols were approved by the local standing committee and authority on animal research. We obtained 6–8 week-old male Sprague–Dawley rats. Rats were divided into 3 groups: the non-treated control group (NT, n = 5), the isoproterenol (ISO)-induced heart failure group without exposure to AP (ISO, n = 8), and the ISO-induced heart failure group with exposure to AP (ISO + AP, n = 5). For inducing heart failure, ISO group rats (ISO and ISO + AP, n = 13) were injected with 75 mg/kg/day of ISO consecutively for 2 days. After 2 weeks, 5 rats (ISO + AP) that had been injected with ISO were subsequently exposed to ambient AP under real urban conditions for 4 weeks (4 h/day, 5 days/week) in the congested traffic roadside of Shinchon-dong, Seoul. The remaining 8 rats (ISO) that had been injected with ISO were considered to be a second control group and ISO and NT groups were subsequently exposed to relatively clean rural air of Hoegi-dong, Seoul. Transthoracic echocardiography (TTE) was performed to evaluate heart function in all rats at baseline and after exposure. All rats were sacrificed to harvest thoracic aorta segments after the second round of TTE. In vitro reactive oxygen species (ROS) and reactive nitrogen species (RNS) assay, tissue malondialdehyde (MDA), total nitric oxide (NO) levels and aortic ring sprouting assay were performed by standard protocol [6]. To analyze and utilize the ambient traffic pollutants under real urban conditions, specially-modified vehicle, mobile emission laboratory (MEL) equipment was designed and modified by researchers (Fig. 1). Unlike other stationary-phase AP measurement devices, MEL is a portable-phase tool that makes it possible to measure AP without being constrained by location. Considering the space utilization and drivability, a commercial mini-van (2400 mm in length, 1100 mm in width, and 1260 mm in height) was selected and modified to contain the MEL equipment. Gas and fine particles from an urban area were provided by the equipment of the mini-van to the Sprague–Dawley rats positioned in exposure chamber (Model: SIG-T, Sibata Scientific Technology LTD., Saitama, Japan). The rats in this chamber were exposed to air at an intake flow rate of 10 L min^{-1}. The inlet gas and ultra-fine particles were piled up and measured by a fast mobility particle sizer and a condensation particle counter. Data are expressed as the mean ± SEM of each measurement. For multiple comparisons between group means, 1-way ANOVA followed by Bonferroni's post-hoc test were performed. The analysis was performed using

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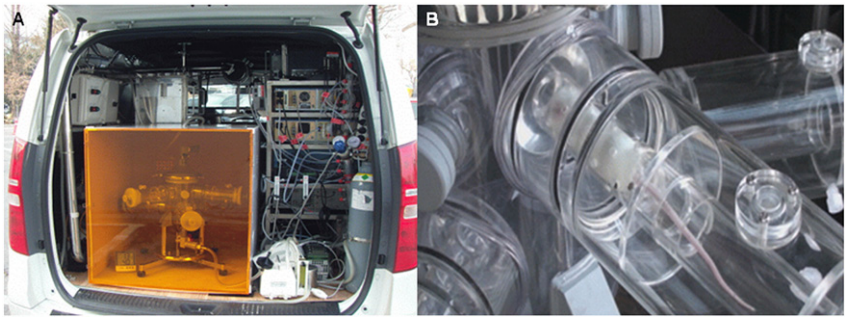

Fig. 1. Real-time photos of the mobile emission laboratory (MEL).

Table 1
Compositions of ambient air pollutants.

	Particle numbers	BCs	PAHs	Surface area	CO	NO	NO_2	NO_x
	(particles cm^{-3})	($\mu g\ m^{-3}$)	($ng\ m^{-3}$)	($\mu m^2\ cm^{-3}$)	(ppm)	(ppb)	(ppb)	(ppb)
Control	20,000 ± 5900	4.1 ± 0.8	19.0 ± 7.6	34.9 ± 5.4	0.6 ± 0.0	24.0 ± 17.0	47.4 ± 16.1	70.2 ± 23.3
Real urban area	69,500 ± 34,000	7.0 ± 3.15	59.0 ± 19.0	57.0 ± 31.0	0.4 ± 0.21	132.0 ± 44.0	42.0 ± 11.5	171.5 ± 51.5

PM, particulate matter; BCs, black carbons; PAHs, polycyclic aromatic hydrocarbons; CO, carbon monoxide; NO, nitrogen monoxide; NO_2, nitrogen dioxide; NO_X, nitrogen oxides.

the statistical software package, SPSS (version 18.0, Chicago, IL, USA). A $p < 0.05$ was considered to be statistically significant.

The distribution of the average concentration of pollutants (particulate and gaseous phases) with meteorological data is depicted in Table 1. The mean EF at baseline was not different statistically between each group. Mean EF measured after 1 month was significantly lower in the ISO group (65.2 ± 8.6%, $p = 0.04$) and in the ISO + AP group (61.6% ± 6.2%, $p = 0.02$) compared with their baseline EF, but it was not different in the NT group (68.1 ± 5.7%, $p = 0.06$) compared with its baseline EF. Intergroup analysis showed no statistical significance ($p = 0.31$). Fluorescent DCF level was higher in the ISO and ISO + AP groups (1749.9 ± 905.9 and 2277.0 ± 729.5 nmol/mg aorta, respectively) compared with the NT group (1577.6 ± 303.5 nmol/mg aorta). For every value in the NT group, the relative

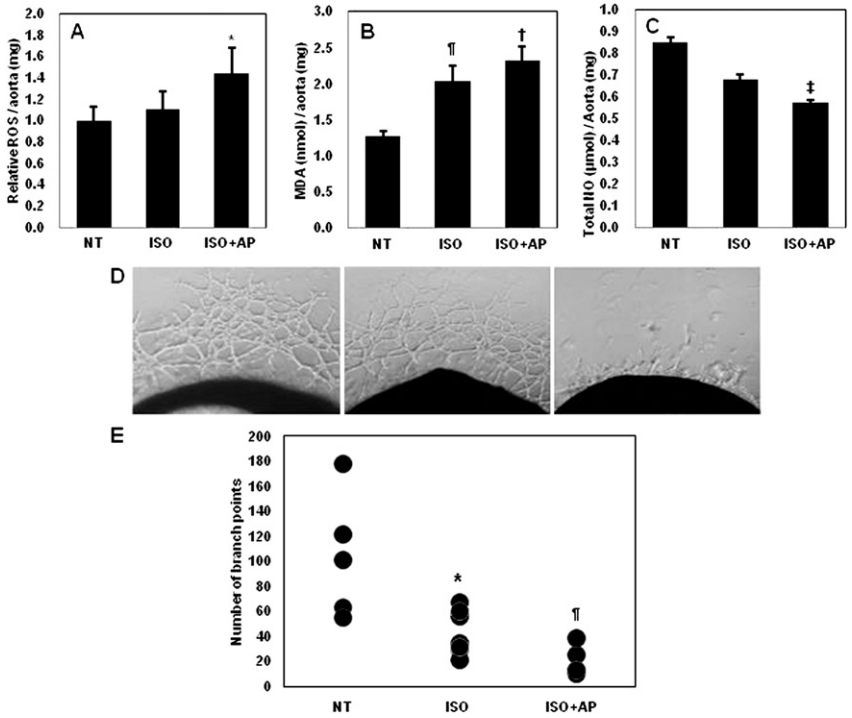

Fig. 2. Results of oxidative stress markers and aortic ring assay. Relative ROS generation (A), MDA (B), and Total NO concentration (C) in homogenates of AP-exposed aortic tissue were presented. Bar graph shows mean ± SEM ($^*p = 0.03$, $^¶p = 0.08$, $^†p = 0.01$, and $^‡p = 0.04$ compared with the NT group). Representative images of aortic ring assay (D). Total numbers of branch points were reduced by 64% and 79% in the ISO and ISO + AP groups compared with the NT group, respectively ($^*p = 0.01$, $^¶p = 0.01$) (E).

ROS concentration of the ISO and ISO + AP groups was increased by 1.11- and 1.44-fold, respectively ($p = 0.03$, Fig. 2A). Tissue MDA concentration was higher in the ISO + AP group (2.3 ± 0.4 nmol/mg aorta) compared with the NT group (1.3 ± 0.1 nmol/mg aorta, $p = 0.01$, Fig. 2B). The total NO level in the ISO and ISO + AP groups (0.7 ± 0.2 nmol/mg aorta and 0.6 ±0.2 nmol/mg aorta, respectively) was lower than that in the NT group (0.9 ± 0.1 nmol/mg aorta), with significant differences between the ISO + AP and NT groups ($p = 0.04$, Fig. 2C). As shown in Fig. 2D, rats belonging to the ISO and ISO + AP groups had impaired microtubule formation in the aorta, and diminished capillary sprouting from the edge of the ring in the AP-exposed aorta. Total numbers of branch points were reduced by 58% and 81% in the ISO and ISO + AP groups, respectively ($p = 0.01$ and 0.01, respectively, Fig. 2E).

AP exacerbated endothelial dysfunction in rat aortas with heart failure under real urban conditions. Furthermore, AP demonstrated significantly attenuated neo-microvascular formation in the aorta compared with the rats belonging to the NT group. Increased ROS generation is thought to be a major cause of excessive oxidative stress production, and impairment of endothelial-dependent vascular homeostasis. A number of previous studies supposed that inhaled PMs caused oxidative stress and production of ROS, which is involved in the pathogenesis of cardiovascular diseases, including hypertension, atherosclerosis, and endothelial dysfunction [7,8]. A majority of these studies were performed under artificial laboratory conditions using condensed PM components to confirm their findings. Instead, we focused on whether similar results could be determined under real urban conditions. In order to fulfill these conditions, we performed our study with MEL equipment which can be used for on-road rat inhalation studies to determine the adverse effects of breathing aerosol in an authentic urban setting. It is thought that deterioration of AP is accelerated by the increased use of fuel-efficient diesel vehicles, which emit large amounts of DEP and toxic gaseous compounds. As presented in Table 1, the average concentrations of PM and NO_2 measured in the roadside of Shinchon-dong were higher than the recommended values determined by the World Health Organization (WHO). Therefore, ambient AP under real urban conditions also may induce oxidative stress via ROS generation or lipid peroxidation, as confirmed in numerous past studies. Our study'smajor limitation is that there is no control group exposed to AP which is important to the interpretation of the effects of AP per se in comparison with the effects of AP in heart failure rats. Further animal- and human-based studies should be performed to establish the exact mechanisms of AP-induced endothelial dysfunction.

There is no potential conflict of interest.

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