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Toxicological Effects of Polycyclic Aromatic Hydrocarbon Quinones Contaminated in Diesel Exhaust Particles

Yoshito Kumagai[1),*] **and Keiko Taguchi**[1),2)]

[1)]Doctoral Programs in Medical Sciences, Graduate School of Comprehensive Human Sciences,
[2)]JST-ERATO Environmental Project, Center for TARA, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan

*Corresponding author. Tel: +81-29-853-3133, E-mail: yk-em-tu@md.tsukuba.ac.jp

ABSTRACT

Accumulated epidemiological and animal studies have suggested that prolonged exposure to ambient particulate matter (PM) is associated with an increased risk of cardiovascular disease and pulmonary dysfunction. While diesel exhaust particles (DEP) contain large variety of compounds, polycyclic aromatic hydrocarbons (PAHs) are a dominant component contaminated in DEP. This article reviews effects of two PAH quinones, 9,10-phenanthraquinone (9,10-PQ) and 1,2-naphthoquinone (1,2-NQ), on vascular and respiratory systems.

Key words: Polycyclic aromatic hydrocarbon, Quinone, Oxidative stress, Vascular dysfunction, Diesel exhaust particle

1. INTRODUCTION

Diesel exhaust particles (DEP) are a major constituent of ambient particulate matter. A variety of organic compounds, such as aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and heterocyclics are found in DEP (Wise *et al.*, 2000; Simoneit *et al.*, 1991; Ramdahl, 1985; Schuetzle, 1983; Schuetzle *et al.*, 1981; Yu and Hites, 1981). The small size of DEP makes them easily inhaled, raising health concerns of their effects on lung cancer, allergy, asthma etc. (Nel *et al.*, 2001; McClellan, 1987). It is well recognized that oxidative stress caused by DEP chemicals plays a critical role in these adverse effects (Xiao *et al.*, 2003; Gavett and Koren, 2001; Takizawa *et al.*, 2000; Li *et al.*, 2000; Hiura *et al.*, 1999; Lim *et al.*, 1998; Sagai *et al.*, 1993). Consistent with this notion, it was recently reported that there were organic chemicals showing redox activity in the airborne particulate matter (Cho *et al.*, 2005). For this reason, identification of the substances in DEP which are involved in oxidative stress through excess amount of reactive oxygen species (ROS) is of interest for elucidating oxidative stress-dependent DEP toxicity.

Sagai *et al.* (1993) reported previously that formation of lung edema following intratracheal DEP injection into mice was markedly suppressed by pretreatment with polyethylene glycol-modified SOD (Sagai *et al.*, 1993). They (Nagashima *et al.*, 1995) subsequently showed that the DEP exposure resulted in the formation, in the mouse lung of 8-hydroxydeoxyguanosine, which is produced by hydroxyl radical (Kasai and Nishimura, 1984). These findings suggested that ROS such as hydroxyl radical derived from superoxide could be enzymatically and continuously generated from DEP and therefore overwhelm defenses. In 1997, we proposed the possibility that DEP components with a quinoid structure, undergo one-electron reduction by pulmonary NADPH-cytochrome P450 reductase to yield semiquinone radical which, in turn, can generate hydroxyl radical through a metal-catalyzed Haber-Weiss reaction through the redox-based generation of superoxide (Kumagai *et al.*, 1997). Therefore, it is likely that such an overproduction of ROS contributes to, at least in part, the lung edema formation and 8-hydroxydeoxyguanosine production observed *in vivo* after exposure to DEP as shown in Fig. 1.

PAH quinones with an α, β-unsaturated carbonyl group are electrophiles that react readily with nucleophiles such as protein thiolates to cause alkylation of crucial cellular proteins (Bolton *et al.*, 2000; Monks *et al.*, 1992; O'Brien, 1991). Alternatively, PAH quinones are highly redox active molecules that can be involved in the redox cycle with their semiquinone radical anions, resulting in the formation of ROS (Squadrito *et al.*, 2001; Bolton *et al.*, 2000; Penning *et al.*, 1996). The current consensus is that PAH quinones are potential candidates for DEP-mediated oxidative stress (Baulig *et al.*, 2003; Xiao *et al.*, 2003; Nel *et al.*, 2001; Li *et al.*, 2000). DEP and diesel exhaust have been reported to contain a variety of PAH quinones such 9,10-phenanthraquinone (9,10-PQ), anthraquinone (AQ), naphthoquinone (NQ), naphtha-

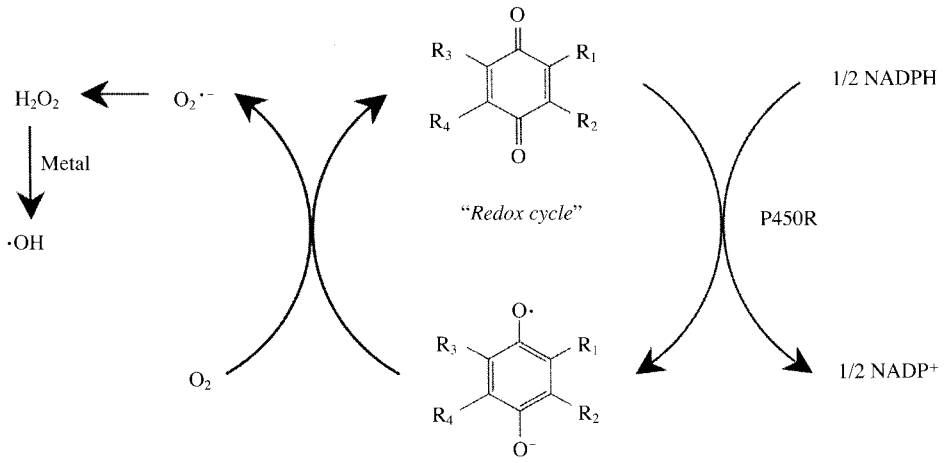

Fig. 1. Metabolic activation of PAH quinones contaminated in DEP to generate reactive oxygen species. P450R, cytochrome P450 reductase.

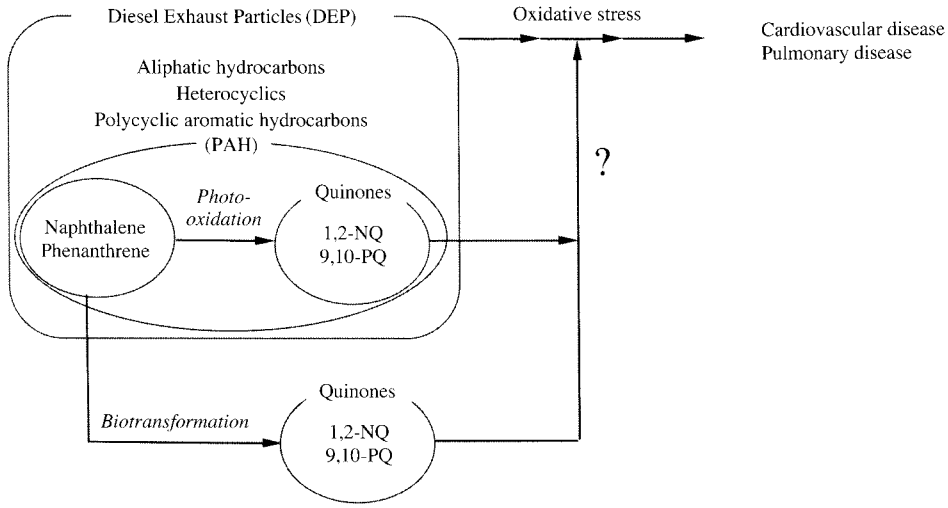

Fig. 2. PAH quinones contaminated in DEP cause oxidative-related diseases.

cenequinone, benzopyrenequinone, crysenequinone and fluorenequinone and possibly these derivatives (Cho *et al.*, 2004; Leotz-Gartziandia *et al.*, 2000; Allen *et al.*, 1997; Schuetzle, 1983). We developed a quantitative method for determination of PQ, 9,10-AQ, 1,2-NQ and 1,4-NQ by electron impact gas chromatography-mass spectrometory (GC-MS) using selected ion monitoring after conversion of the quinones in the samples to their stable diacetyl derivatives (Cho *et al.*, 2004). Using this procedure, the mean concentrations of 9,10-PQ, 9,10-AQ, 1,2-NQ, and 1,4-NQ were 24, 40, 14 and 8 μg per g of DEP (donated by Dr. H. Takano, National Institute for Environmental Studies, Japan), respectively. These results suggest that the levels of quinoid compounds in DEP are fairly low. However, our recent immunochemical study with specific antibody against 1,2-NQ revealed that naphthalene and its analogs, abundant combustion products in volatile-phase of the atmosphere (Fraser *et al.*, 1998), are extensively biotransformed by enzyme systems in the body to form NQs (T. Miura *et al.*, unpublished observation) and that the reactive quinones are covalently bound to macromolecules associated with irreversible toxicity (Zheng *et al.*, 1997).

It should be, therefore, noted that the metabolic activation of PAHs (Smithgall *et al.*, 1988) to quinones as well as photooxidation contributes to atmospheric quinone exposure (Squadrito *et al.*, 2001) as shown in Fig. 2. In this article, the pathophysiological and toxicological actions of 9,10-PQ and 1,2-NQ (see Fig. 3) examined by our laboratory are reviewed.

2. DECREASED NITRIC OXIDE (NO) PRODUCTION AND MECHANISMS INVOLVED

Epidemiologic studies have suggested that expo-

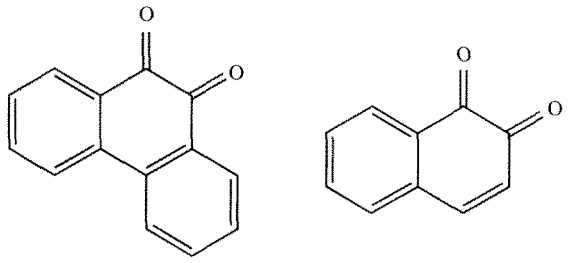

Fig. 3. Structures of 9,10-phenanthraquinone (9,10-PQ) and 1,2-naphthoquinone (1,2-NQ).

sure of humans to ambient particulate matter is associated with cardiopulmonary-related diseases and mortality (Pope *et al.*, 2002, 1995; Dockery *et al.*, 1993). NO is a biological signalling molecule that plays an important role in neurotransmission, vasorelaxation and immune response (Moncada *et al.*, 1991) and is synthesized from L-arginine by NO synthase (NOS). All NOS isozymes consist of an *N*-terminal oxygenase domain and a *C*-terminal reductase domain, which is highly homologous with NADPH-cytochrome P450 reductase; this domain is capable of transferring electrons from NADPH to artificial acceptor molecules such as PAH quinones. Thus, we hypothesized that quinoid compounds could interact with the reductase domain on NOS, leading to a decrease in NO formation from L-arginine. Using rat cerebellar enzyme preparations that contained neuronal NOS, we showed that the inhibition of NO formation by quinones which exhibit a one-electron reduction potential (E^1,7) ranging between −240 and −90 mV, increased at a more positive E^1,7 (Kumagai *et al.*, 1998b). Among 15 PAH quinones tested, 9,10-PQ (E^1,7 = −124 mV) and 1,2-NQ (E^1,7 = −89 mV), inhibited NO production most potently (IC_{50} value = 10-12 μM) (Kumagai *et al.*, 1998b). With purified neuronal NOS, we found that this enzyme effectively reduced the 9,10-PQ and 1,2-NQ, thereby causing a marked decrease in the production of NO from L-arginine (Kumagai *et al.*, 1998b). In contrast, 9,10-AQ (E^1,7 = −348 mV) which showed negligible inhibitory effects on neuronal NOS activity, was not reduced by the enzyme. Taken together, we concluded that 9,10-PQ interacts with the P450 reductase domain on neuronal NOS, and inhibits NO formation by shunting electrons away from the normal catalytic pathway.

Ikeda *et al.* (1995) reported previously that incubation of rat aortic rings with suspensions of DEP resulted in a suppression of endothelium-dependent

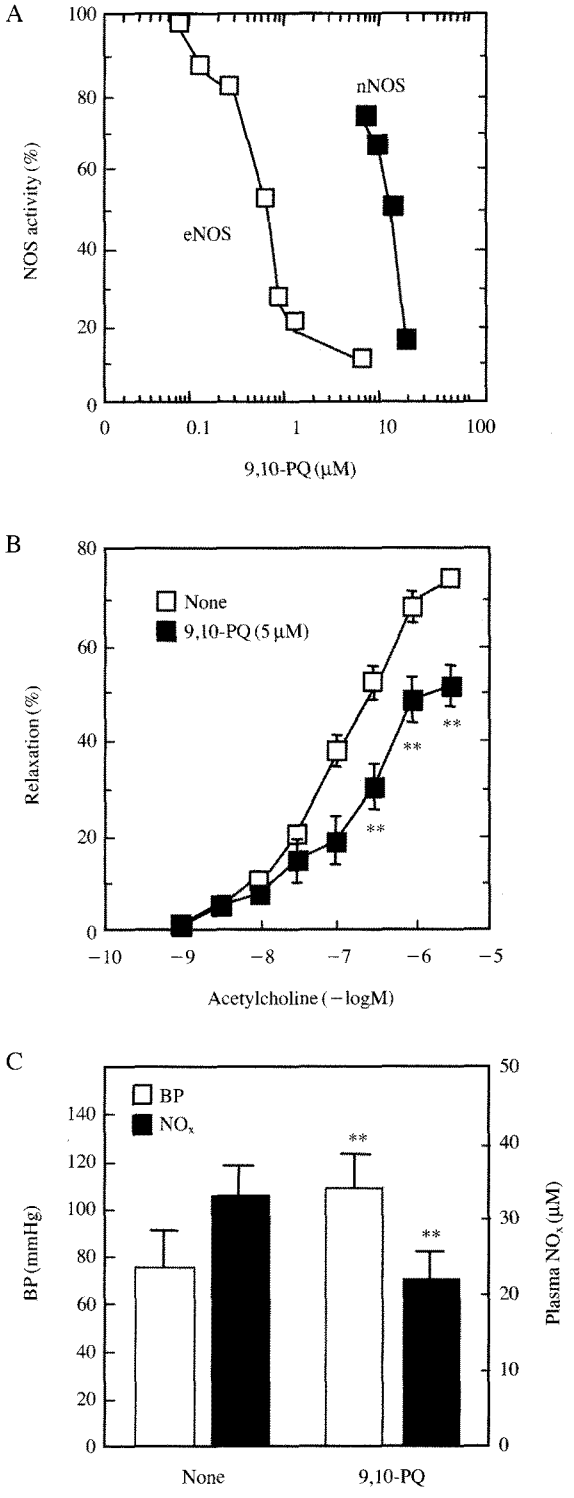

Fig. 4. Changes in catalytic activities of NOS isoforms (A), endothelium-dependent relaxation of rat aorta (B), mean blood pressure in rats and plasma NO_x levels in rats (C) by 9,10-PQ. 9,10-PQ, 9,10-phenanthraquinone; BP, blood pressure; NO_x, nitric oxide metabolites (as an index for systemic NO production *in vivo*); nNOS, neuronal NOS; eNOS, endothelial NOS. **, $P<0.01$ vs. controls.

vasorelaxation caused by acetylcholine, suggesting that DEP components in urban air contribute to a disruption of vasorelaxation. Since impairment of NO production in the endothelium is implicated in the pathophysiology of vascular diseases (Kelly *et al.*, 1996; Umans and Levi, 1995), we hypothesized that 9,10-PQ could inhibit the enzymatic activity not only of neuronal NOS but also of endothelial NOS, thereby altering NO formation, which could lead to suppression of endothelial NOS-dependent vasorelaxation and increased blood pressure. In a study using the total membrane fraction of bovine aortic endothelial cells, 9,10-PQ was found to be a more potent inhibitor of endothelial NOS than of neuronal NOS, with an IC_{50} value of 0.6 μM (Fig. 4A) (Kumagai *et al.*, 2001). Endothelium-dependent relaxation of rat aortic rings by acetylcholine was significantly suppressed by 9,10-PQ (5 μM) (Fig. 4B). Moreover, exposure of rats to 9,10-PQ (0.36 mmol/kg) by intraperitoneal administration resulted in an elevation of blood pressure (1.4-fold). Under these conditions, systemic NO production, evaluated by plasma levels of NO metabolites in rats given 9,10-PQ, was reduced to 68% of control levels (Fig. 4C). These findings were consistent with the notion that 9,10-PQ and compounds like it, could be components of DEP that affect endothelial NOS activity, thereby suppressing NO-dependent vascular tone. To further substantiate this notion, we have recently reported that 1,2-NQ was a potent inhibitor of endothelial NOS as well (IC_{50} value=1.4 μM) and thus cause a profound suppression of acetylcholine-dependent vasorelaxation at a concentration of 5 μM (Sun *et al.*, 2006).

3. REDOX CYCLING OF 9,10-PQ AND OXIDATIVE STRESS

As mentioned earlier, quinones have two chemical characteristics, 1) covalent bond forming ability by electrophilic attack on nucleophilics, leading to thiol adduct formation and/or 2) redox cycling, in which their rapid reduction and oxidation results in generation of ROS (Bolton *et al.*, 2000; Monks *et al.*, 1992; O'Brien, 1991). Our studies indicated that 9,10-PQ reacts easily with dithiol compounds without loss of 9,10-PQ (Kumagai *et al.*, 2002), indicating that the reactivity of 9,10-PQ toward dithiols is due to redox cycling. Such an interaction of dithiol with PAH quinones, resulting in thiol oxidation, was seen with 1,2-NQ, 1,4-NQ, 2,3-dichloro-1,4-NQ and juglone, whereas little appreciable oxidation was seen with 2-anilino-1,4-NQ, lapachol, 9,10-AQ and 5,12-naphthacenequinone. These results suggested that 9,10-PQ-mediated oxidation of proximal protein thiols and the reduction of molecular oxygen is involved in the destruction of cellular protein sulfhydryls.

Sugimoto *et al.* (2005) reported that exposure of human pulmonary epithelial A549 cells to 9,10-PQ induced apoptosis with a LC_{50} of ~7 μM. Oxidation of the cellular protein as determined by formation of protein carbonyls was also detected in cells after treatment with 9,10-PQ, suggesting that 9,10-PQ induces oxidative damage, presumably through generation of ROS. Interestingly, treatment of A549 cells with 10-20 μM 9,10-PQ for 12 h specifically down-regulated protein levels of Cu,Zn-superoxide dismutase and heme oxygenase-1 by more than 50% (Sugimoto *et al.*, 2005). Thus, it seems likely that 9,10-PQ causes oxidative stress through not only redox cycling but also disruption of antioxidant defense system.

4. TRACHEAL CONTRACTION CAUSED BY 1,2-NQ AND MECHANISMS INVOLVED

The exacerbation of asthma is one of the major adverse health effects of ambient particulate matter (PM). In studies of 1,2-NQ, we found that this quinone also mediates the contraction of tracheal smooth muscle but the effects were persistent. These persistent effects suggested that affected pulmonary tissue would become resistant to dilation, resulting in exacerbation of the asthma syndrome. Based on these considerations, we explored, *ex vivo*, the underlying mechanisms for this effect (Kikuno *et al.*, 2006). We found that 1,2-NQ is capable of causing a concentration dependent contraction of tracheal smooth muscle in guinea pigs with EC_{50} value of 18.7 μM (Fig. 5A). At a concentration of 50 μM, the contraction intensities of 1,2-NQ, 1,4-NQ and 1,4-BQ were 75%, 63%, 11% of maximum response to 40 mM KCl, respectively, indicating a structural selectivity of the effect (Fig. 5B). The tricyclic quinones, 9,10-PQ, 9,10-AQ, and 5,12-naphthacenequinone, were without an effect on tracheal contraction even at 500 μM. Other polycyclic aromatic hydrocarbons such as naphthalene, anthracene, 1,2-benzanthracene, 2,3-benzofluorene, benzo[a]pyrene, chrysene, dibenzofuran, 3,6-dimetylphenanthrene, fluoranthrene, 2-nitropyrene, phenanthrene and pyrene did not affect tracheal tension under these conditions (Fig. 5B). These findings suggest that the tracheal contraction involves covalent attachment of the quinone group to protein cysteine residues (Zheng *et al.*, 1997). Several lines of evidence suggested that 1,2-NQ activated phos-

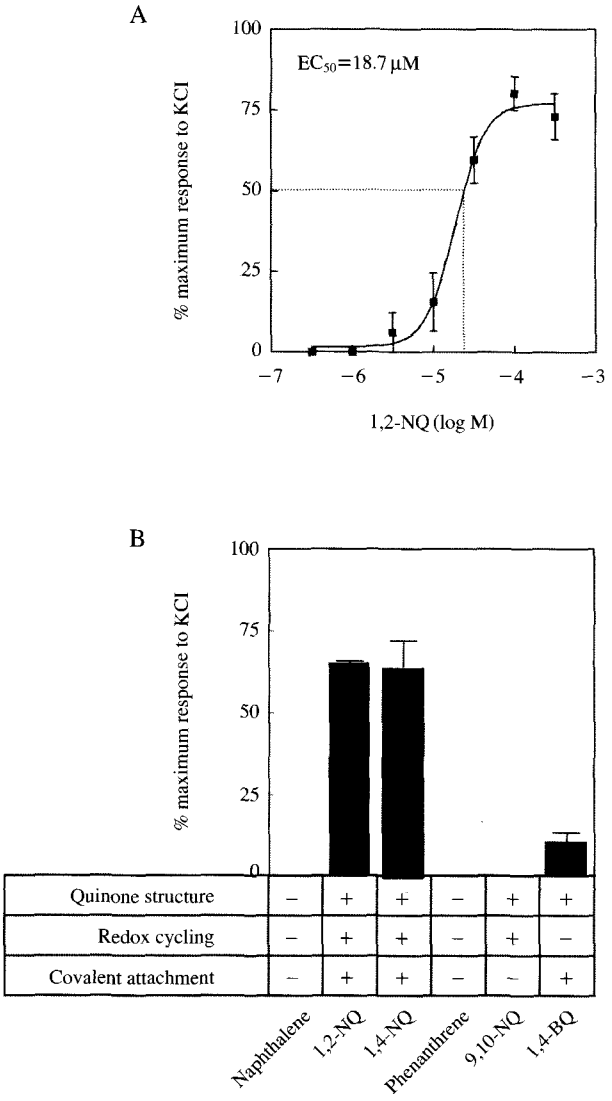

Fig. 5. Tracheal contraction of guinea pig *ex vivo*. (A) Concentration-dependent tracheal contraction by 1,2-NQ. (B) Chemical property-related tracheal contraction of PAHs and their quinones.

pholipase A2/lipoxygenase/vanilloid receptor signaling. More interestingly, 1,2-NQ was capable of transactivating protein tyrosine kinases (PTKs) such as epidermal growth factor receptor (EGFR) in guinea pig trachea, suggesting that phosphorylation of PTKs contributes to 1,2-NQ-induced tracheal contraction. *In vitro* study with A549 cells and cell-free system with purified protein tyrosine phosphatase 1B (PTP1B) have revealed that the transactivation of EGFR caused by 1,2-NQ exposure is, at least in part, attributable to inactivation of PTP1B through covalent binding of 1,2-NQ to PTP1B (N. Iwamoto *et al.*, unpublished observation).

5. PULMONARY TOXICITY OF PAH QUINONES *IN VIVO*

Takano and his associates reported previously that DEP enhance airway inflammation in mice (Ichinose *et al.*, 1998; Takano *et al.*, 1998, 1997). It was also reported that DEP exposure is associated with allergic inflammation and increased immunoglobulin levels (Casillas *et al.*, 1999; Nel *et al.*, 1998; Diaz-Sanchez *et al.*, 1994). However, it remained to be identified which component (s) from DEP are responsible for such an action. When 9,10-PQ (1 μg/body) was intratracheally injected into mice, macrophage levels in the bronchoalveolar lavage fluid (BALF) were not significantly different from those of control animals, but there was evidence for induction of neutrophils and eosinophils 24 hr after injection of 9,10-PQ. At 48 hr, the levels of neutrophils and eosinophils in 9,10-PQ-exposed group were approximately 10.6 times ($P<0.05$) and 71.9 times ($P<0.05$), respectively, of those in control groups (Hiyoshi *et al.*, 2005a). Under the conditions, 9,10-PQ exposure caused a significant enhancement of pulmonary expression of interleukin-5 (IL-5, 5.6-fold) and eotaxin (1.9-fold), but not IL-1β, IL-2, IL-4, GM-CSF, IFN-γ, TNF-α, KC, MIP1-α or MCP-1 in the lungs (Hiyoshi *et al.*, 2005a). These findings suggest that intratracheal exposure of mice to 9,10-PQ by single administration induces recruitment of inflammatory cells through the local expression of IL-5 and eotaxin. In the presence of ovalbumin (OVA), 9,10-PQ (2.1 ng/body) significantly increased the numbers of eosinophils and mononuclear cells in BALF as compared to OVA alone (Hiyoshi *et al.*, 2005b). In contrast, the numbers of these cells around the airways were not significantly different between OVA challenge and OVA plus 9,10-PQ challenge in lung histology. 9,10-PQ exhibited adjuvant activity for the OVA-specific production of IgG_1 and IgE (Hiyoshi *et al.*, 2005b). These observations indicated that 9,10-PQ can enhance immunogloblin production and the infiltration of inflammatory cells into alveolar spaces that are related to OVA.

We also investigated the effects of 1,2-NQ on antigen-related airway inflammation, local expression of cytokine proteins, and antigen-specific IgGs production in mice (Inoue *et al.*, 2007). Intratracheal administration of 1,2-NQ into mice aggravated antigen-related airway inflammation characterized by infiltration of eosinophils and lymphocytes around the airways and an increase in goblet cells in the bronchial epithelium in a dose-dependent manner. Combined exposure to 1,2-NQ and antigen enhanced

the local expression of IL-4, IL-5, eotaxin, MCP-1, and KC as compared with exposure to antigen or 1,2-NQ alone. It was also found that 1,2-NQ exhibited adjuvant activity for the antigen-specific production of IgG_1 and IgG_{2a}. Overall, it is suggested that 9,10-PQ and 1,2-NQ are PAH quinones that enhance antigen-related airway inflammation *in vivo*.

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