Cardiovascular and lung inflammatory effects induced by systemically administered diesel exhaust particles in rats

Abderrahim Nemmar,1 Sultan Al-Maskari,2 Badreldin H. Ali,3 and Issa S. Al-Amri4

Departments of 1Physiology, 2Dean’s Office, 3Pharmacology, and 4Pathology, Electron Microscopy Unit, College of Medicine and Health Sciences, Sultan Qaboos University, Al-Khod, Sultanate of Oman

Submitted 26 June 2006; accepted in final form 26 October 2006

Nemmar A, Al-Maskari S, Ali BH, Al-Amri IS. Cardiovascular and lung inflammatory effects induced by systemically administered diesel exhaust particles in rats. Am J Physiol Lung Cell Mol Physiol 292: L664–L670, 2007. First published November 3, 2006; doi:10.1152/ajplung.00240.2006.—Pollution by particulates has consistently been associated with increased cardiorespiratory morbidity and mortality. It has been suggested that ultrafine particles, of which diesel exhaust particles (DEP) are significant contributors, are able to translocate from the airways into the bloodstream in vivo. In the present study, we assessed the effect of systemic administration of DEP on cardiovascular and respiratory parameters. DEP were administered into the tail vein of rats, and heart rate, blood pressure, blood platelet activation, and lung inflammation were studied 24 h later. Doses of 0.02, 0.1, or 0.5 mg DEP/kg (8, 42, or 212 μg DEP/rat) induced a significant decrease of heart rate and blood pressure compared with saline-treated rats. Although the number of platelets was not affected, all the doses of DEP caused a shortening of the bleeding time. Similarly, in addition to triggering lung edema, the bronchoalveolar lavage analysis revealed the presence of neutrophil influx in DEP-treated rats in a dose-dependent manner. We conclude that the presence of DEP in the systemic circulation leads not only to cardiovascular and haemostatic changes but it also triggers pulmonary inflammation.

Numerous epidemiological studies reported consistent associations between exposures to particulate air pollution with a diameter ≤10 μm (PM10) and cardiorespiratory mortality and morbidity (3, 40, 41). These studies found associations between particulate matter and hospital admissions for various cardiovascular diseases, including congestive heart failure (41, 42) and coronary heart disease (37). Also, an increased risk for acute myocardial infarction (31, 32) and cardiorespiratory symptoms (19) have been reported in association with particulate air pollution.

The strongest associations were found for fine particles with a diameter <2.5 μm (PM2.5) and that have an important role in triggering pathophysiological changes (31, 40). These particles, and particularly the ultrafine fraction (<100 nm), of which the combustion-derived particulates of diesel exhaust are an important component, penetrate deeply into the respiratory tract and can carry large amounts of toxic compounds, such as hydrocarbons and metals, on their surfaces (7).

Currently, different lines of particle-related research are being pursued (2, 25, 29, 46). It has been suggested that inhaled particles may lead to pulmonary inflammation and subsequent release of soluble mediators that may influence blood coagulation parameters (8). The autonomic nervous system may also be a target for the adverse effects of air pollution (10). We (23, 28) and others (9, 18, 30, 44) have reported extrapulmonary translocation of ultrafine particles (UFP) after intratracheal instillation or inhalation, suggesting an alternative and/or a complementary explanation for the cardiovascular effects of particles. However, the mechanisms related to the cardiorespiratory effects of translocated particles are not well known.

We recently reported in hamsters that DEP lead to a significant prothrombotic tendency, activation of circulating blood platelets, as well as lung inflammation as early as 1 h and persisting up to 24 h (22, 24, 27). Pulmonary inflammation and peripheral thrombosis were correlated at 6 and 24 h, but the prothrombotic tendency observed 1 h after DEP exposure did not appear to correlate with pulmonary inflammation (27). The latter is compatible with direct platelet activation by DEP, having presumably penetrated into the circulation (9, 18, 30, 44).

To circumvent the effects related to pulmonary accumulation of particles and release of inflammatory mediators, several studies adopted a pharmacodynamic approach consisting of administering a precise amount of particles intravascularly. It has been shown that within 1–2 h after their systemic administration, UFP cause prothrombotic effects in the femoral vein of hamsters (26), ear vein of rats (43), and the hepatic microvasculature of mice (16). However, the direct effect of particles on cardiovascular endpoints and pulmonary inflammation is not known.

Therefore, the aim of this study was to investigate, in vivo, the acute (24 h) effects of systemic administration of DEP on heart rate, blood pressure, and hemostasis, and to assess whether and to which extent these effects are associated with the development of pulmonary inflammation.

Materials and Methods

Particles. We used diesel exhaust particles (DEP; SRM 2975) from the National Institute of Standards and Technology (NIST; Gaithersburg, MD). DEP were suspended in sterile saline (NaCl 0.9%) containing Tween 80 (0.1%). To minimize aggregation, particle suspensions were always sonicated (Clifton Ultrasonic Bath, Clifton, NJ) for 15 min and vortexed before their dilution and before intravenous administration. Control animals received saline containing Tween 80 (0.1%).

For electron microscopy, droplets (10 μl) of a suspension of 1 mg of DEP in 500 μl were placed on matured Formvar/carbon film for

Address for reprint requests and other correspondence: A. Nemmar, Sultan Qaboos Univ., College of Medicine and Health Sciences, Dept. of Physiology, PO Box 35, Muscat 123, Al-Khod, Sultanate of Oman (e-mail: anemmar@squ.edu.om).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
RESULTS

Particle characterization. Transmission electron microscopy of the DEP showed numerous small aggregates of carbonaceous particles less than 100 nm. Most of these aggregates were <1 μm in the largest diameter (Fig. 1).

Effect of DEP on blood pressure. The systemic administration of DEP induced a significant decrease of blood pressure in DEP-exposed rats at doses of 0.02 (−28 mmHg, P < 0.05), 0.1 (−32 mmHg, P < 0.05), and 0.5 mg/kg (−24 mmHg, P < 0.05) compared with mean blood pressure observed in saline-treated rats (Fig. 2).

Effect of DEP on heart rate. Figure 3 shows that the administration of DEP at doses of 0.02, 0.1, and 0.5 mg/kg, in rats, resulted in a significant reduction of the heart rate to 348 ± 13 (P < 0.05), 348 ± 8 (P < 0.05), and 339 ± 12 beats per minute (bpm) (P < 0.05) compared with 389 ± 11 bpm recorded in saline-treated rats.

Effect of DEP on tail bleeding time. Figure 4A illustrates a shortening of the tail bleeding time in rats exposed to 0.02, 0.1, and 0.5 mg/kg of DEP. The shortening, which has been shown to be platelet dependent (13, 33, 45), was significant at the dose of 0.02 (305 ± 17 s, P < 0.01), 0.1 (283 ± 55 s, P < 0.01), and (255 ± 35 s, P < 0.01) 0.5 mg/kg compared with the control group (533 ± 63 s). Platelet counts in blood did not significantly increase following DEP administration (Fig. 4B).

Effect of DEP on WBC and RBC numbers. No significant effect of DEP at the doses of 0.02, 0.1, and 0.5 mg/kg on the numbers of granulocytes, monocytes, or lymphocytes compared with saline-treated rats (Fig. 5).

Similarly, the numbers of RBC were not significantly affected by the DEP administration compared with the control group (Fig. 5).

Effect of DEP on pulmonary inflammation. Depending on the systemic treatment performed, the cells found in BAL were primarily macrophages and polymorphonuclear neutrophils (PMN) (Fig. 6). Macrophages were not found in control rat BAL. No other cells were observed microscopically.

The systemic administration of DEP resulted in a marked cellular influx in the lung at doses of 0.02, 0.1, and 0.5 mg/kg. Although it did not reach statistical significance, the number of macrophages increased at the dose of 0.5 mg/kg (Fig. 6A). Figure 6B shows that the PMN numbers increased significantly at 0.02 mg/kg (2.9 ± 0.3 × 10⁴/ml, P < 0.05), 0.1 mg/kg (3.4 ± 0.4 × 10⁴/ml, P < 0.05), and 0.5 mg/kg (5.6 ± 1.2 × 10⁴/ml, P < 0.001) compared with saline-treated rats (0.9 ± 4.0 × 10⁴/ml).

Wet-to-dry weight ratio. Figure 7 shows the results of the lung wet-to-dry weight ratio. A significant increase of this relationship was observed following the administration of the doses of 0.02, 0.1, and 0.5 mg/kg of DEP (P < 0.05) compared with saline-treated rats.

DISCUSSION

In this study, we provide evidence that the systemic administration of DEP in the circulation affects the blood pressure, heart rate, and hemostasis 24 h later. We have also demonstrated that the presence of such particles in the circulation trigger pulmonary edema and lung inflammation evaluated by BAL fluid analysis.
Exposure of human subjects to DEP results in an acute inflammatory response characterized by neutrophil and mast cell influx into the airways (38, 39). Moreover, it has been demonstrated that DEP impairs the regulation of vascular tone and endogenous fibrinolysis (20). We recently showed in hamsters that pulmonary exposure to DEP causes lung inflammation and enhances the occurrence of arterial and venous thrombosis and that these effects persisted up to 24 h (22, 27). Pretreating hamsters with diphenhydramine, a histamine H1 receptor antagonist, strongly reduced lung inflammation at all time points investigated (i.e., 1, 6, and 24 h). Such pretreatment reduced the thrombotic events at 6 and 24 h but not at 1 h after DEP administration. The findings at 1 h are compatible with direct platelet activation by DEP, having presumably penetrated the systemic circulation. However, the effects observed at 6 and 24 h are related to lung inflammation. We have also confirmed that anti-inflammatory pretreatment can abrogate the peripheral thrombogenicity by preventing histamine release from mast cells and PMN influx in the lung (24). Because we have achieved the inhibition of pulmonary inflammation and peripheral thrombosis by intraperitoneal injection of dexamethasone, diphenhydramine, or cromoglycate, we may well have inhibited the effect of DEP that have translocated into the systemic circulation. Moreover, at the 24-h time point, when we pretreated hamsters with intratracheal instillation of dexamethasone, before exposing them to DEP, the observed inhibition of both PMN influx in the lung and thrombosis was only partial (24). Thus it is plausible to hypothesize that the translocated DEP (and their associated constituents) that have presumably occurred within 1 h could contribute in the observed pulmonary and extrapulmonary effects at 24 h.

Consequently, in the present study, we wanted to investigate whether and to what extent the presence of DEP in the systemic circulation can trigger cardiovascular and pulmonary inflammatory changes at 24 h. To this end, we used an admittedly less physiological mode of administration, namely, the intravascular route, because we wanted to mimic the effect of inhaled particles translocated from the lungs into the systemic circulation (9, 14, 15, 23, 28, 30). The advantage of this approach is that it circumvents effects related to the pulmonary accumu-
tion of particles, e.g., release of inflammatory mediators, and it allows us to study, in vivo, the direct effect of DEP on the heart, hemostasis, and whether the presence of particles in blood can contribute in the development of lung inflammation.

The electron microscopy analysis of the DEP used in the present study revealed the presence of a substantial amount of ultrafine (nano)-sized particle aggregates (Fig. 1). These particles are comparable to the DEP (NIST; SRM 1650) we previously used (22, 24, 27). Therefore, it seems reasonable to postulate passage of these particles as it has been demonstrated to occur (9, 14, 15, 23, 28, 30). The lowest dose of DEP used in the present study, i.e., 8 μg/rat, can presumably be achieved in the blood after pulmonary exposure to 32–100 μg/rat (28, 30).

To minimize aggregation, particles were always sonicated for 15 min and vortexed immediately (<1 min) before their dilution in saline containing Tween 80 (0.1%) as well as before intravascular administration. Although the electron microscopy analysis clearly demonstrated the presence of UFP and larger particle aggregates (<1 μm in largest diameter), we do not know how much of the total injected dose consists of UFP or larger aggregates and whether the observed effects are caused by UFP or larger aggregates.

Fig. 2. Blood pressure in Wistar Kyoto rats is shown 24 h after the systemic administration of saline or DEP. Means ± SE (n = 6–7). Statistical analysis was by Newman-Keuls test.

Fig. 3. Heart rate in Wistar Kyoto rats is shown 24 h after the systemic administration of saline or DEP. Means ± SE (n = 6–7). Statistical analysis was by Newman-Keuls test.

Fig. 4. Tail bleeding time (A) and platelet numbers (B) are shown for Wistar Kyoto rats 24 h after the systemic administration of saline or DEP. Means ± SE (n = 6–7). Statistical analysis was by Newman-Keuls test.

Fig. 5. Red blood cells, monocytes, granulocytes, and lymphocytes numbers are shown for Wistar Kyoto rats 24 h after the systemic administration of saline or DEP. Means ± SE (n = 6–7).
Our data show that 24 h following their systemic administration, DEP cause a decrease of blood pressure and heart rate. This effect could be explained by the production of reactive oxygen species in the heart, responsible for cardiac dysfunction (11, 34). Analogous findings have been made in spontaneously hypertensive rats intratracheally instilled with combustion-derived particles in which the decrease of blood pressure and heart rate did not return to preexposure values until 72 and 48 h after dosing, respectively (49). Similarly, it has been shown that the exposure of healthy rats by instillation to PM2.5 or by inhalation to concentrated ambient particles was responsible for a decrease of blood pressure and heart rate within the first and second hour of particle exposure (5, 36). However, others have reported an increase of heart rate in pulmonary hypertensive rats after exposure to concentrated ambient particles (5). Interestingly, these discrepancies seem to confirm the epidemiological observations that found both decrease and increase of blood pressure in relation to air pollution exposures [well reviewed by Delfino et al. (6)]. These disparities were related to the differences between subject populations, type of regional air pollutants, or underlying pathology (healthy, asthma, or chronic heart disease). Additional studies are needed to uncover the pathophysiological mechanisms of particle-induced changes in heart rate and blood pressure.

We have recently shown that DEP enhance arterial and venous thrombosis after intratracheal instillation both in vivo and ex vivo. Moreover, we also reported that DEP induce platelet aggregation in vitro (22, 27). Nevertheless, the effect of systemic administration of DEP has not been addressed. Here, we show that the presence of DEP in the systemic circulation shortens the bleeding time, which has been shown to be platelet dependent (13, 33, 45). Our findings corroborate with previous studies that showed that the intravascular administration of positively charged ultrafine polystyrene particles or carbon black particles are capable to trigger thrombotic complications (16, 26, 43). In agreement to our previous findings (22), the number of platelets did not significantly change following DEP administration. It is likely that DEP, which are taken up by phagocytosis or the open canalicular system of platelets, might predispose them to aggregation and thrombosis (1, 48).

An important finding of our study is that we show that the intravascular administration of DEP causes pulmonary inflammation and edema. In line with these results, diffusional movement of UFP administered intravascularly to the alveolar space has been reported in vivo in rabbits and in an ex vivo model of isolated perfused rabbit lungs (12, 21). We recently reported that pulmonary inflammation and peripheral thrombosis caused by intratracheal instillation of DEP are correlated at 6 and 24 h, but the prothrombotic tendency observed at 1 h resulted from direct platelet activation by DEP, having presumably translocated into the circulation (27). Based on the present results, we suggest that the pulmonary inflammation we previously observed at 24 h after pulmonary deposition of DEP (27) could result, at least partly, from the translocated DEP-associated components or by DEP themselves.

We conclude that the presence of DEP in the systemic circulation leads not only to cardiovascular and hemostatic changes, but it also triggers pulmonary inflammatory reaction. Further studies are needed to establish which constituents are

![Fig. 6. Macrophages (A) and PMN (B) numbers in bronchoalveolar lavage (BAL) fluid are shown for Wistar Kyoto rats 24 h after the systemic administration of saline or DEP. Means ± SE (n = 6–7). Statistical analysis was by Newman-Keuls test.](http://ajplung.physiology.org/)

![Fig. 7. Shown is left lung wet/dry weight ratio per 100 g body wt (BW) in Wistar Kyoto rats 24 h after the systemic administration of saline or DEP. Means ± SE (n = 6–7). Statistical analysis was by Newman-Keuls test.](http://ajplung.physiology.org/)
responsible for the effect of DEP (i.e., the physical and/or chemical properties of DEP) and what mechanism is involved.

REFERENCES


