Toxicology 262 (2009) 50–56

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Evaluation of the direct systemic and cardiopulmonary effects of diesel particles in spontaneously hypertensive rats

Abderrahim Nemmar[a,*], Subramanian Dhanasekaran[a], Javed Yasin[b], Hajer Ba-Omar[c], Mohamed A. Fahim[a], Elsadig E. Kazzam[b], Badreldin H. Ali[d]

[a] Department of Physiology, Faculty of Medicine and Health Sciences, United Arab Emirates University, P.O. Box 17666, Al Ain, United Arab Emirates
[b] Department of Internal Medicine, Faculty of Medicine and Health Sciences, United Arab Emirates University, P.O. Box 17666, Al Ain, United Arab Emirates
[c] Department of Physiology, College of Medicine & Health Sciences, Sultan Qaboos University, P.O. Box 35, Muscat 123, Al-Khod, Oman
[d] Department of Pharmacology and Clinical Pharmacy, College of Medicine & Health Sciences, Sultan Qaboos University, P.O. Box 35, Muscat 123, Al-Khod, Oman

ARTICLE INFO

Article history:
Received 9 March 2009
Received in revised form 6 May 2009
Accepted 7 May 2009
Available online 20 May 2009

Keywords:
Diesel exhaust particles
Lung inflammation
Systemic inflammation
Spontaneously hypertensive rats

ABSTRACT

Recent data suggest that ultrafine pollutant particles (diameter <0.1 μm) may pass from the lung into the systemic circulation. However, the systemic and cardiorespiratory effects of translocated particles are not well known. In this study, we determined the direct acute (24 h) effect of the systemic administration of 0.01 mg/kg and 0.02 mg/kg diesel exhaust particles (DEP) on systolic blood pressure, heart rate, and both systemic and pulmonary inflammation in spontaneously hypertensive rats (SHR). Compared to the blood pressure in control group, rats exposed to DEP exhibited a dose-dependent increase in systolic blood pressure, at 0.01 mg/kg ($P < 0.05$) and 0.02 mg/kg ($P < 0.01$). Likewise, the heart rate was also dose-dependently increased at 0.01 mg/kg (P:NS) and 0.02 mg/kg ($P < 0.01$) compared to control SHR. DEP exposure (0.02 mg/kg) significantly elevated the number of leukocytes in blood ($P < 0.05$), interleukin-6 (IL-6, $P < 0.005$), tumor necrosis factor alpha ($P < 0.05$) and leukotriene B4 (LTB4, $P < 0.005$) concentrations in plasma. Moreover, in SHR given 0.02 mg/kg, the number of platelet was significantly reduced ($P < 0.05$), whereas the tail bleeding time was prolonged ($P < 0.05$). Pulmonary inflammations were confirmed by the presence of a significant increase in the number of macrophages (0.02 mg/kg) and neutrophils (0.01 and 0.02 mg/kg) and protein contents (0.02 mg/kg) in bronchoalveolar lavage (BAL) compared to saline-treated SHR. Also, IL-6 (0.01 mg/kg; $P < 0.05$ and 0.02 mg/kg; $P < 0.01$), LTB4 (0.02 mg/kg; $P < 0.05$) concentrations in BAL and the superoxide dismutase activity (0.02 mg/kg; $P = 0.01$) were significantly elevated compared to control group. We conclude that, in SHR, the presence of DEP in the systemic circulation leads not only to cardiac and systemic changes, but also triggers pulmonary inflammatory reaction involving IL-6, LTB4 and oxidative stress.

© 2009 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Several epidemiological studies have reported that ambient air pollution not only affects the respiratory tract, but also has systemic and cardiovascular effects (Ruckerl et al., 2006; Kunzli et al., 2005). Short-term exposure to particulate air pollution increases the susceptibility to ischemia (Pekkanen et al., 2002) and myocardial infarction (Peters et al., 2001). Exhaust from diesel powered vehicles is a major source of particulate matter (PM) in urban air and the main contributor of ultrafine particles (UFP) with a diameter of less than 100 nm. These UFP have high alveolar deposition and ability to translocate to the bloodstream (Riedl and Diaz-Sanchez, 2005). Acute exposure to DEP in healthy human volunteers causes an inflammatory response in airways characterized by neutrophil and mast cell influx into the airways (Salvi et al., 1999, 2000) and impairs the regulation of vascular tone and endogenous fibrinolysis (Mills et al., 2005).

The mechanisms underlying the cardiopulmonary effect of particulate air pollution have not been fully elucidated (Mills et al., 2009). Recent evidence suggested that UFP, soluble components from the particles, such as metals and organic substances, enter the circulatory system and act on the first encountered target organ such as the heart, liver or brain (Nemmar et al., 2001, 2002a; Oberdorster et al., 2002; Shimada et al., 2006; Chen et al., 2006; Geiser et al., 2005). Besides, it has been shown that inhaled particles elicit inflammatory reactions in the lungs and proinflammatory mediators pass into the systemic circulation, contributing to platelet activation and endothelial dysfunction (Vermylen et al., 2005).

* Corresponding author. Tel.: +971 37137533; fax: +971 3 7671966.
E-mail addresses: anemmar@uaeu.ac.ae, anemmar@hotmail.com (A. Nemmar).

0300-483X/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2009.05.009

Therefore, it is difficult to discriminate between these two processes and to determine the contribution of each pathway. To avoid the effects related to the pulmonary accumulation of particles, e.g., release of inflammatory mediators, and exclusively determine the effect of translocated particles, several studies have assessed the effect of administering defined amount of particles intravascularly (Silva et al., 2005; Nemmar et al., 2002b; Khandoga et al., 2004). Recently, in normotensive Wistar Kyoto (WKY) rats, we provided evidence that 24 h after their systemic administration, DEP (0.5–0.02 mg/kg) affect the blood pressure, heart rate and trigger lung inflammation (Nemmar et al., 2007a). However, the inflammatory mediators involved in the systemic and pulmonary effects have not been identified. Moreover, the direct effects of DEP on cardiovascular endpoints and pulmonary inflammation in animal model of hypertension, namely spontaneously hypertensive rats (SHR) are still unclear. Therefore, the aim of this study is to investigate in SHR the acute (24 h) effects of systemic administration of DEP (0.01 and 0.02 mg/kg) on heart rate, systolic blood pressure, systemic and pulmonary inflammation, and to identify the inflammatory mediators involved in these effects.

2. Materials and methods

2.1. Particles

We used diesel exhaust particles (DEP; SRM 2975) from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). 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, USA) for 15 min and vortexed before their dilution and prior to intravenous administration. Control animals received saline containing Tween 80 (0.1%).

These particles were previously analysed by transmission electron microscopy and showed the presence of a substantial amount of ultrafine (nano)sized particle aggregates, and larger particle aggregates (<1 μm in largest diameter) (Nemmar et al., 2007a).

The endotoxin level, which was semiquantified by the Sigma E-TOXATE (Limulus Amoebocyte Lysate) test, was lower than the detection limit of 0.05 EU/ml in the vehicle and DEP solutions.

2.2. Systemic administration of particles

This project was reviewed and approved by the Institutional Review Board of the United Arab Emirates University, Faculty of Medicine and Health Sciences and experiments were performed in accordance with protocols approved by the Institutional Animal Care and Research Advisory Committee.

Sixteen-week-old male SHR (Taconic Farms Inc., Germantown, NY, USA), weighing 282 ± 7 g were placed in restrainers. The tail was disinfected with ethanol, and 150 μl of vehicle (n = 9) or doses of 0.01 (n = 6) or 0.02 (n = 6) mgDEP/kg corresponding to about 2.8 or 5.6 μg DEP/rat were injected into the tail vein.

Experiments could not be completed in all animals on the same day. However, at least one relevant control animal was always included on each experimental day. Twenty-four hours after systemically injecting rats with DEP, we measured systolic blood pressure and heart rate, tail bleeding time and some constituents in blood and bronchoalveolar (BAL) fluid.

2.3. Systolic blood pressure and heart rate measurements

Heart rate and systolic blood pressure were measured in the conscious restrained SHR using a computerized tail-cuff system (Harvard apparatus; Columbus Instruments) (Nemmar et al., 2007a).

2.4. Bleeding time measurements

To determine the effects of DEP on bleeding time, measurements were performed using a tail-cut model, which was previously shown to be platelet dependent (Kihara et al., 2001). Rats were anesthetized by i.p. administration of a combination of ketamine (60 mg/kg) and xylazine (5 mg/kg). Then the tail was transected about 0.5 cm from the tip using a disposable surgical blade. The tail was placed in 25 ml isotonic saline (pH 7.4, 37 °C) immediately after being cut and the bleeding time was measured from the moment of transection until bleeding stopped completely.

2.5. Blood collection and BAL fluid analysis

In the same animals, immediately after measuring the bleeding time, blood was drawn from the inferior vena cava in EDTA (4%). A sample was used for platelets, white blood cells (WBC) and red blood cells (RBC) counts using an ABX Micros 60 counter (ABX Diagnostics, Montpellier, France). The remaining blood was centrifuged during 15 min at 3500 rpm, and the plasma samples were stored at −80 °C.

The rats were killed by an overdose of ketamine, and BAL was then performed by cannulating the trachea. The lungs were lavaged three times with 7 ml sterile 0.9% NaCl. The BAL fluid was pooled in a plastic tube on ice. No difference in the amount of recovered fluid was observed between the different groups. BAL fluid was centrifuged (1000 × g × 10 min, 4 °C). Counting of the cells was performed in a hemocytometer after resuspension of the pellets and staining with 1% gentian violet. The cell differentials were performed on cytocentrifuge preparations fixed in methanol and stained with Diff Quick (Dade Behring, Marburg, Germany). The supernatant was stored at −80 °C until further analysis.

2.6. Determination of total protein, interleukin-6 (IL-6), tumour necrosis factor alpha (TNFα) leukotriene B4 (LTB4) and superoxide dismutase (SOD) activity in BAL supernatant and plasma

In BAL supernatant and plasma, IL-6, TNFα and LTB4 concentrations were determined using ELISA commercial kits (Pierce, Thermo Scientific, Rockford, IL, USA, Quantikine TNFα, R&D Systems, USA and Cayman Chemical, Ann Arbor, MI, USA, respectively).

Total proteins were measured in BAL supernatant using commercial kit (Biorad München, Germany) (Nemmar et al., 2003a).

The SOD activity, used as a marker of BAL oxidative stress, was measured using a commercial kit (Randox, Antrim, UK).

2.7. Statistics

Data are expressed as means ± SD. All data were analyzed with GraphPad Prism Version 4.01 for Windows software (Graphpad Software Inc., San Diego, USA). Data were analyzed for normal distribution using the D'Agostino and Pearson omnibus normality test. Blood pressure and heart rate (normally distributed) were analysed using parametric analysis of variance with a Dunnett's *post hoc* test. The data not normally distributed were analyzed by 2 × 2 column comparison via a non-parametric Mann–Whitney two-tailed test. P-values < 0.05 were considered to be significant.

3. Results

3.1. Effect of DEP on systolic blood pressure and heart rate in SHR

Fig. 1a illustrates the effect of systemic administration of DEP on systolic blood pressure in SHR. Compared to blood pressure in control SHR, rats exposed to DEP exhibited a dose-dependent increase in systolic blood pressure, at 0.01 mg/kg (P < 0.05) and 0.02 mg/kg (P < 0.01). Likewise, Fig. 1b shows that the heart rate was also dose-dependently increased, but the level of significance was only reached with 0.02 mg/kg (P < 0.01) compared to control SHR.

3.2. Effect of DEP on leukocyte numbers in blood and IL-6 and LTB4 in plasma

Although the level of significance was only reached at 0.02 mg/kg (P < 0.05), DEP induced a dose-dependent increase in the number of leukocytes compared to saline treated SHR (Fig. 2a). The numbers of RBC were not affected by the exposure to 0.01 ($7.99 \pm 0.5 \times 10^6$/μl blood) or 0.02 ($8.04 \pm 1.2 \times 10^6$/μl blood) mg/kg DEP compared to the control group ($8.04 \pm 0.4 \times 10^6$/μl blood).

Similarly, the occurrence of systemic inflammation was confirmed by the significant increase of LTB4 (P < 0.005), IL-6 (P < 0.005) and TNFα (P < 0.05) in plasma of SHR systemically administered with 0.02 mgDEP/kg compared to SHR treated with saline (Fig. 2b–d).

3.3. Effect of DEP on platelet numbers and bleeding time

The systemic administration of DEP induced a dose-dependent decrease in platelet numbers at 0.01 mg/kg (−13.5%; P:NS) and 0.02 mg/kg (−24.5%; P < 0.05), compared to control group (Fig. 3a). Fig. 3b illustrates a prolongation of the tail bleeding time in SHR exposed to 0.01 mg/kg (+21%; P:NS) and 0.02 mg/kg (+87%; P < 0.05), compared to control SHR.

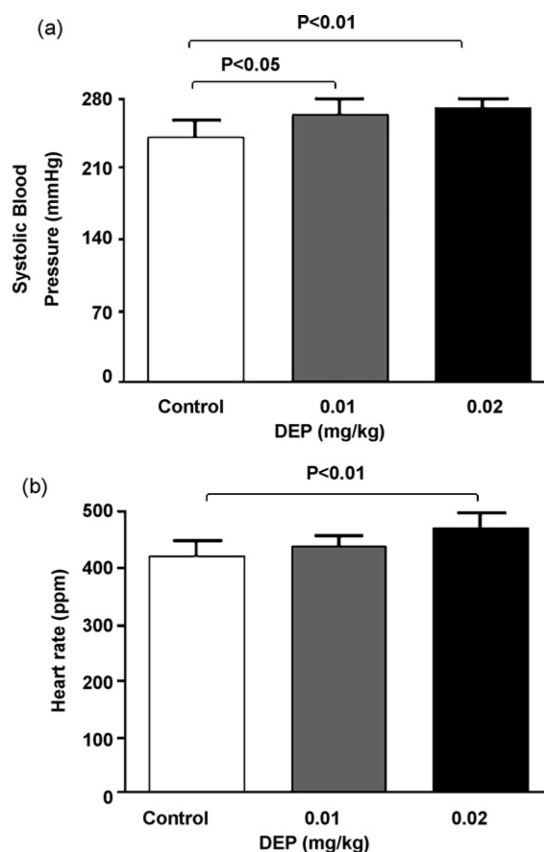

Fig. 1. Systolic blood pressure (a) and heart rate (b) in spontaneously hypertensive rats, 24 h after the systemic administration of saline, 0.01 or 0.02 mg/kg diesel exhaust particles. Data are mean ± SD (n = 6–9). Statistical analysis is by one-way ANOVA followed by Dunnett's multiple-comparison test.

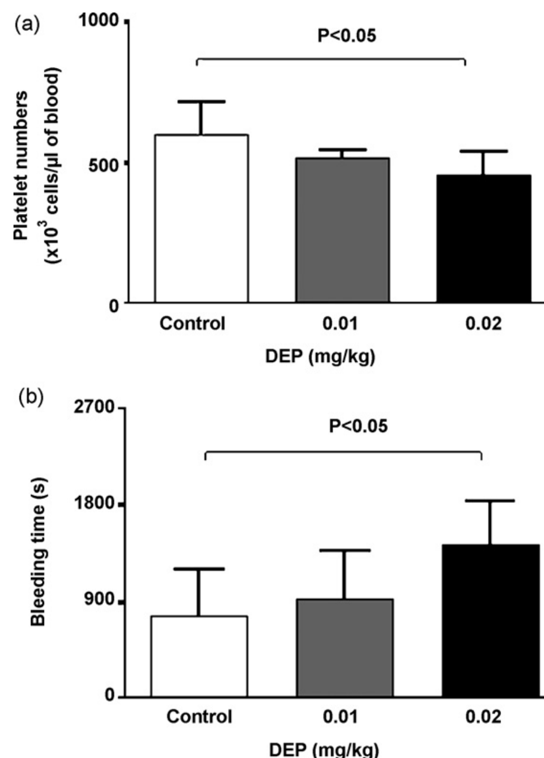

Fig. 3. Platelet numbers (a) and tail bleeding time (b) in spontaneously hypertensive rats, 24 h after the systemic administration of saline, 0.01 or 0.02 mg/kg diesel exhaust particles. Data are mean ± SD (n = 6–9). Statistical analysis by 2 × 2 column comparison via a two-tailed Mann–Whitney test.

3.4. Effect of DEP on cellular and proteins content in the BAL

The systemic administration of DEP resulted in a cellular influx in the lung (Fig. 4a and b). The number of macrophages increased significantly following DEP exposure (0.02 mg/kg, $P < 0.05$), compared to saline-treated SHR (Fig. 4a). Fig. 4b shows that the PMN numbers increased following exposure to 0.01 ($P < 0.05$) and 0.02 ($P < 0.005$) mg DEP/kg, compared to saline-treated SHR.

The total protein concentrations in BAL, which was used as a marker of increase in pulmonary permeability, was also signifi-

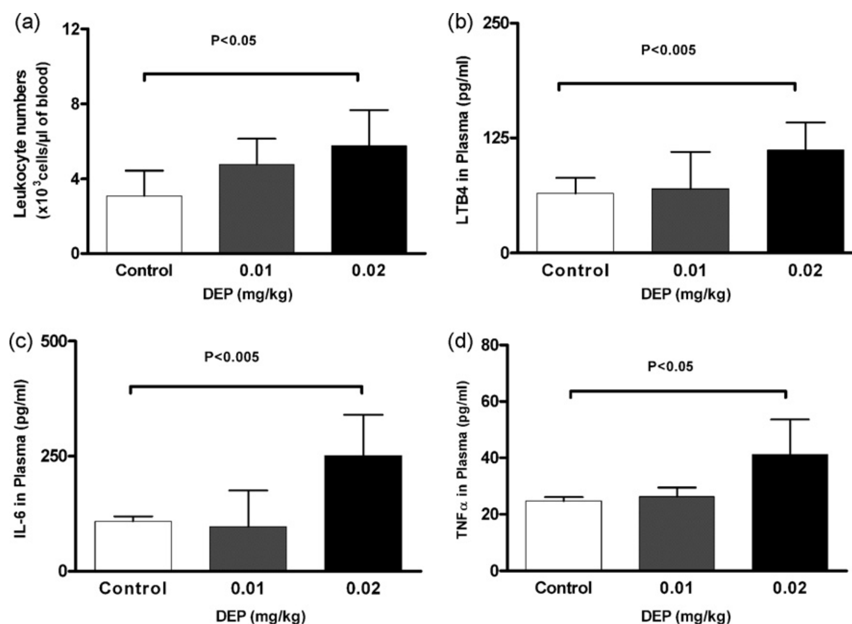

Fig. 2. Leukocyte numbers in blood (a), leukotriene B4 (LTB4, b), interleukin 6 (IL-6, b) and tumor necrosis factor alpha (TNFα, d) in plasma in spontaneously hypertensive rats, 24 h after the systemic administration of saline, 0.01 or 0.02 mg/kg diesel exhaust particles. Data are mean ± SD (n = 6–9). Statistical analysis by 2 × 2 column comparison via a two-tailed Mann–Whitney test.

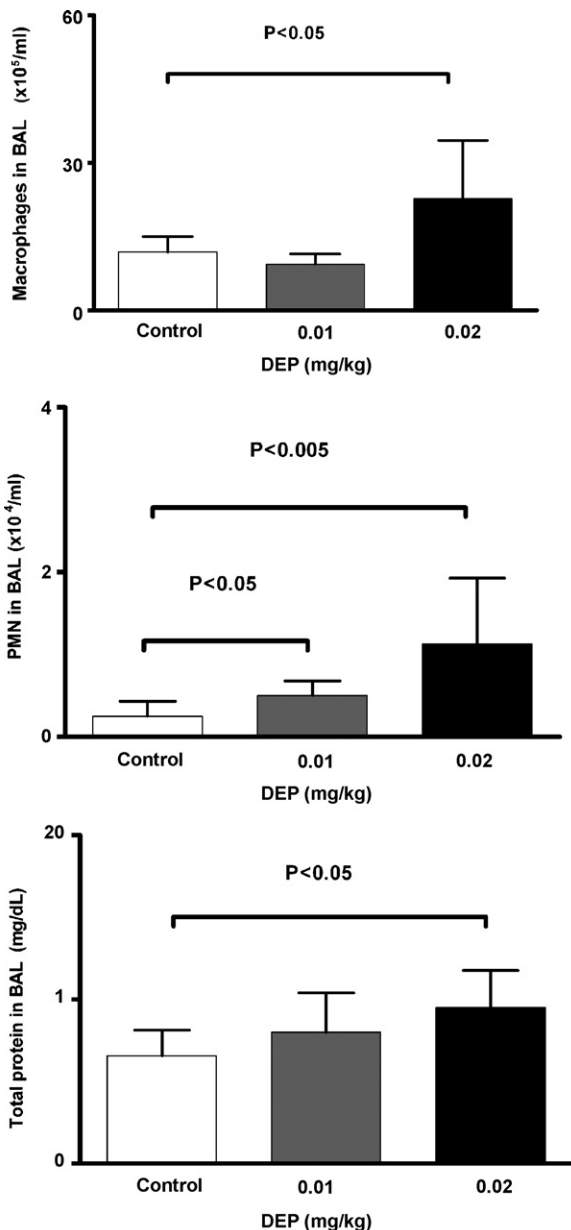

Fig. 4. Macrophage (a) and PMN (b) numbers, and total protein concentrations (c) in bronchoalveolar lavage (BAL) fluid in spontaneously hypertensive rats, 24 h after the systemic administration of saline, 0.01 or 0.02 mg/kg diesel exhaust particles. Data are mean ± SD (n = 6–9). Statistical analysis by 2 × 2 column comparison via a two-tailed Mann–Whitney test.

cantly increased following the systemic administration of 0.02 mg DEP/kg (P < 0.05) compared to saline in SHR (Fig. 4c).

3.5. Effect of DEP on BAL levels of IL-6, LTB4 and SOD

The effect of the vascular administration of DEP on the concentrations of IL-6, TNFα, LTB4 and SOD activity in BAL is illustrated in Fig. 5. The presence of pulmonary inflammation was substantiated by the significant increase of IL-6 following exposure to 0.01 (P < 0.05) and 0.02 (P < 0.01) mgDEP/kg (Fig. 5a). TNFα concentrations slightly but insignificantly increased following exposure to DEP (0.02 mg/kg, Fig. 5b). The concentration of LTB4 was significantly increased following the administration of 0.02 mg/kg (P < 0.05, Fig. 4c).

Correspondingly, Fig. 5d shows that the SOD activity in BAL was increased in SHR exposed to DEP (0.02 mg/kg, P = 0.01) compared to saline-exposed group.

4. Discussion

In the present study, we provide evidence that 24 h post-treatment with DEP, its presence in the circulation of SHR significantly increased the systolic blood pressure, heart rate and prolonged bleeding time. We have also established the occurrence of systemic and pulmonary inflammation and identified the involvement of IL6, TNFα, LTB4 and SOD.

Urban air pollution consists of an extremely complex mixture of gaseous and particulate agents. Among others, diesel exhausts contain gaseous (SO_2, NO_2, and CO) and particulate components, all of which have been shown to be associated with increased cardiovascular morbidity and mortality, including the occurrence of acute myocardial infarction (Brook et al., 2004; Mills et al., 2009). Since air pollutants affect the human body simultaneously, rather than separately, further studies are needed also to verify the impact of pollution inhalation, as a whole, on human health.

Although it is well established the exposure to particulate air pollution increase cardiopulmonary morbidity and mortality, the mechanisms underlying these effects have not been fully elucidated (Mills et al., 2009). As inhaled particles leads to lung inflammation with release of inflammatory mediators (Vermylen et al., 2005; Nemmar et al., 2003b, 2004b), and particle translocation (Chen et al., 2006; Nemmar et al., 2001, 2002a; Oberdorster et al., 2002, 2004; Elder et al., 2006; Shimada et al., 2006), consequently, it is, hard to distinguish between these two processes. To specifically investigate the effect of translocated particles, we recently demonstrated, in normotensive WKY rats, that 24 h following their systemic administration, DEP (0.02–0.05 mg/kg) caused a decrease in heart rate and blood pressure, shortening of bleeding time and caused pulmonary inflammation assessed by bronchoalveolar lavage (Nemmar et al., 2007a). Therefore, in the present study, we continued to systemically administer DEP but in animal model of hypertension and assessed whether and to which extent are the effects of DEP different in SHR. We also attempted identify the inflammatory mediators that are involved in these effects. In order to allow comparison with our previously published study (Nemmar et al., 2007a) in normotensive WKY rats, we purposefully studied the same time point, and, because of their relevance, we selected the lowest dose (0.02 mg/kg) that we have previously tested (Nemmar et al., 2007a), and even a lower dose (i.e. 0.01 mg/kg). Particle translocation from the airways into the circulation may occur directly or after ingestion by alveolar macrophages (Mills et al., 2009; Nemmar et al., 2004b). Although extra-pulmonary translocation of ultrafine particles after intratracheal instillation or inhalation has been reported, the amount of ultrafine particles that translocates into blood and extra-pulmonary organs differed among these studies (Nemmar et al., 2004b). Recently, Mutlu et al. (2007) reported that pulmonary exposure to 35 μg of particulate matter for a mouse (25 g in weight) triggers IL-6 production by alveolar macrophages, resulting in reduced clotting times, intravascular thrombin formation, and accelerated arterial thrombosis. This dose corresponds to a pulmonary exposure to 395 μg of particulate matter for a rat (weighing 282 g). The doses of particles that we injected systemically in the present study, i.e. 0.01 mg/kg (2.8 μg per rat) and 0.02 mg/kg (5.6 μg per rat), represents 0.7% and 1.4% of the pulmonary administered dose (395 μg/SHR).

We previously analyzed DEP used in the present study by transmission electron microscopy, and confirmed the presence of a substantial amount of ultrafine-sized aggregates (Nemmar et al., 2007a). These particles are comparable to other type of DEP that we (Nemmar et al., 2003a, 2004a) and others (Boland et al., 1999) have

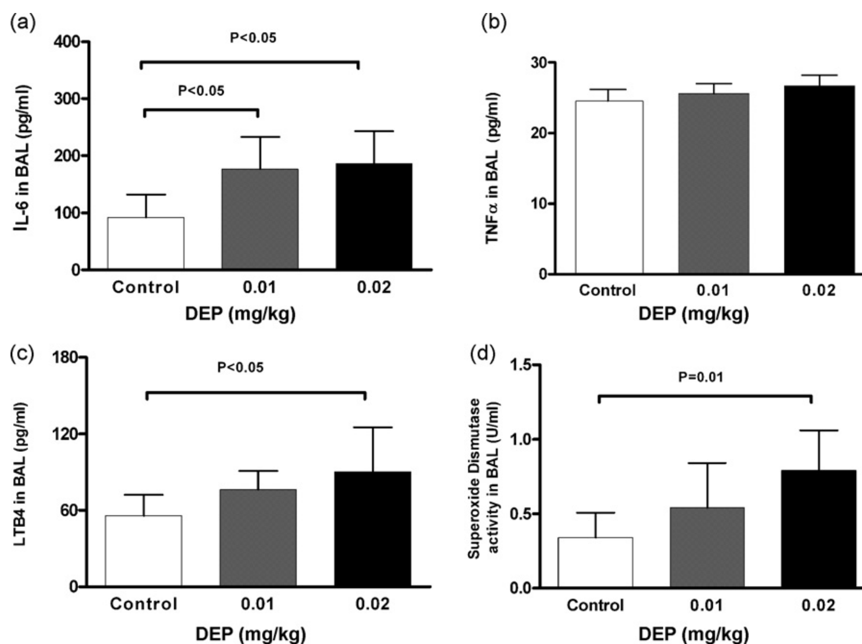

Fig. 5. Interleukin 6 (IL-6, a), tumor necrosis factor alpha (TNFα, b), leukotriene B4 (LTB4, c) and superoxide dismutase (SOD, d) levels in bronchoalveolar lavage (BAL) fluid in spontaneously hypertensive rats, 24 h after the systemic administration of saline, 0.01 or 0.02 mg/kg diesel exhaust particles. Data are mean ± SD (n = 6–9). Statistical analysis by 2 × 2 column comparison via a two-tailed Mann–Whitney test.

previously used *in vivo* and *in vitro*. Accordingly, it is reasonable to postulate passage of these particles as it has been demonstrated to occur (Nemmar et al., 2001, 2002a; Oberdorster et al., 2002; Geiser et al., 2005; Kapp et al., 2004; Kato et al., 2003). Our data show that 24 h following their administration, DEP increased blood pressure and heart rate. Similar findings have been reported in pulmonary hypertensive rats after exposure by inhalation to concentrated ambient particles or during dust storm events (Chang et al., 2004, 2007). At present, the precise pathophysiologic mechanisms of the blood pressure increase remain speculative. It has been suggested that induction of systemic oxidative stress (Gurgueira et al., 2002; Urch et al., 2005) and a proinflammatory response (Salvi et al., 1999) may alter the bioavailability of nitric oxide within the vasculature and/or by increasing several vasoconstrictive factors, such as endothelin (Brook et al., 2002; Peretz et al., 2008). Moreover, an imbalance of the autonomic nervous system favoring an increase in sympathetic drive (Gold et al., 2000) may also contribute to this hypertensive response.

In contrast, in normotensive WKY rats, we previously reported that 24 h after DEP administration (0.02–0.5 mg/kg), the blood pressure and heart rate were decreased (Nemmar et al., 2007a). Others (Rodriguez Ferreira Rivero et al., 2005) also showed that the exposure of healthy rats by instillation to $PM_{2.5}$ was responsible for a decrease of blood pressure and heart rate within the first and second hour of particle exposure. These differences could be related to the underlying disease and suggest that the effect of particulate air pollution can not only be exacerbated in diseased conditions but also be different in people with pre-existing cardiopulmonary diseases. Likewise, epidemiological observations found both decrease and increase in blood pressure in relation to air pollution exposures and these differences were explained by the type of air pollutants or underlying diseases (Delfino et al., 2005).

Pulmonary exposure to pollutant particles can provoke an acute systemic thrombogenic response associated with pulmonary injury/inflammation and oxidative stress in SHR (Kodavanti et al., 2002). Interestingly, our data show that the intravascular administration of DEP to SHR causes a significant increase in the number of leukocytes in blood, indicating the occurrence of systemic inflammation. Moreover, we found that the IL-6 and TNFα concentrations were significantly increased following DEP exposure. Both IL-6 and TNFα are proinflammatory cytokines. Moreover, their levels have been correlated with risk factors for cardiovascular disease such fibrinogen, white blood cell count, and blood viscosity (Welsh et al., 2008). With respect to particulate air pollution, recently, Dubowsky et al. (2006) reported epidemiological evidence for positive associations between air pollution and indicators of systemic inflammation such as leukocyte numbers, CRP and IL-6. They also found that the associations with IL-6 was strongest for individuals with diabetes, obesity or hypertension (Dubowsky et al., 2006). In addition to IL-6 and TNFα, we also found an increase in LTB4 in plasma. LTB4 is an eicosanoid lipid derivative of the arachadonic acid signaling pathway generated by 5-lipoxygenase and leukotriene A4 hydrolase. LTB_4 is a potent chemoattractant that facilitates recruitment and endothelial cell adhesion of neutrophils to the inflammatory site and promotes recruitment of inflammatory cells into tissues (Hicks et al., 2007). Taken together, the increase of leukocyte numbers and IL-6, TNFα and LTB4 concentrations in plasma indicate the occurrence of systemic inflammation which could explain the increase of blood pressure and heart rate (Lee et al., 2004). Although we did not find an effect of DEP on RBC counts, it is reasonable to presume that erythrocytes, the main cells for number and size in blood, may be the first target encountered by the systemically administered DEP. It has been suggested that erythrocyte dysfunctions, considered as a key feature of cardiovascular disease, may represent potential source of systemic inflammation (Zappulla, 2008). Indeed, oxidized erythrocytes may promote the systemic release, from activated macrophages, of IL-6 and TNFα which, by activating the hormonal stress response, may induce adaptations such as blood pressure increase (Zappulla, 2008). Further studies are needed to clarify this point.

We have recently shown that exposure to nanoparticulates enhance thrombosis *in vivo*, *ex-vivo* and *in vitro* (Nemmar et al., 2002b, 2003a,b, 2004a, 2005, 2008). Besides, we found that 24 h after the systemic administration of DEP (0.02–0.5 mg/kg) to normotensive WKY rats, there was a significant shortening of the bleeding time, suggesting that platelet aggregation has occurred

(Nemmar et al., 2007a). However, the number of platelets was not affected by DEP exposure (Nemmar et al., 2007a). The present data show a significant prolongation of the bleeding time. This observation could not be considered necessarily an antithrombotic effect of DEP in SHR. Indeed, 24 h following the administration of DEP, the number of circulating platelets dropped in a dose dependent manner. No effect of DEP exposure has been observed at earlier time point, i.e. 6 and 12 h, and a statistically insignificant trend toward a decrease in the number of platelets has been observed at 18 h after DEP injection (not shown). The significant decrease of platelet numbers observed at 24 h time point pleads in favor of an aggregating effect which could be related either to the direct effect of DEP on circulating platelet (Nemmar et al., 2003a), and/or as consequence of systemic inflammation and release proagreggatory inflammatory mediators such as platelet activating factor or IL-6. In the present study, we found that IL-6 increased both in the systemic circulation and bronchoalveolar lavage, and it is known IL-6 plays a pivotal role in hemostasis (Mutlu et al., 2007). Therefore, we speculate that aggregated platelets are removed from the circulation and accumulate in various organs including the lung (Zarbock et al., 2006; Kasperska-Zajac and Rogala, 2006). The loss of circulating platelets is manifested by the prolongation of rat tail bleeding time, which has been shown to be platelet dependent (Kihara et al., 2001). We have recently reported a prolongation of the tail bleeding time in mice exposed to carbon nanotube (Nemmar et al., 2007b). This effect was not interpreted as antithrombotic, as carbon nanotube caused increase in thrombosis in the carotid artery (Nemmar et al., 2007b). Additional studies involving the examination of arterial and venous thrombosis model are needed to clarify this issue.

In the present study, we confirm the occurrence of lung inflammation by the influx of macrophage (0.02 mg DEP/kg) and PMN (0.01 and 0.02 mg DEP/kg), and the increase of total protein, indicative of increased epithelial permeability. We have recently made similar observations after pulmonary and systemic exposure to DEP in healthy animals (Nemmar et al., 2003a,b, 2004a, 2007a). Moreover, similarly to the systemic findings, the concentrations of IL-6 and LTB4 were increased in BAL. Because of their chemotactic activities (Martin et al., 1989; Fielding et al., 2008), these increases could explain the inflammatory cell recruitment in BAL. To our knowledge, the link between particulate air pollution and LTB4 has not been reported before. However, IL-6 concentrations have been found to increase after exposure to particulate air pollution *in vivo* and *in vitro* (Mutlu et al., 2007; Bayram et al., 1998).

The role of the oxidative stress in particulate-induced toxicity in different organs such as lung, liver and heart is well established (Bhatnagar, 2006). However, its occurrence in lung following the systemic exposure particulate exposure to DEP is not known. To determine whether pulmonary oxidative stress was caused by DEP exposure, SOD activity was determined in BAL. SOD is one the most important antioxidant enzymes responsible for the oxidative balance in the lungs (Rahman et al., 2006). Our data show that DEP induced an increase in SOD activity indicating an *in vivo* response of the lung to an oxidant challenge. Elevations of SOD activity has been reported in BAL of patients with coal workers' pneumoconiosis (Ulker et al., 2008), BAL of mice exposed to cigarette smoke (Valenca et al., 2008) and in rat lungs following exposure to particles (Ghio et al., 1994).

We conclude that the presence of DEP in the systemic circulation leads to systemic inflammation characterized by leukocytosis and an increase in IL-6, TNFα and LTB4 concentrations in plasma. Blood pressure and heart rate were also increased by DEP. Moreover, we showed that DEP caused pulmonary inflammation, and identified the involvement of IL-6, LTB4 and oxidative stress. Our data illustrate that translocated particles can not only affect the cardiovascular parameters, but also potentiate pulmonary inflammation. Additional studies are needed to establish which constituents of DEP (nanoparticles or chemical constituents adsorbed onto them) are responsible for these effects.

Conflict of interest statement

None.

Acknowledgement

This work was supported by funds of the United Arab Emirates University grant (Grant number: 01-05-8-11/08).

References

Bayram, H., Devalia, J.L., Sapsford, R.J., Ohtoshi, T., Miyabara, Y., Sagai, M., Davies, R.J., 1998. The effect of diesel exhaust particles on cell function and release of inflammatory mediators from human bronchial epithelial cells in vitro. Am. J. Respir. Cell Mol. Biol. 18, 441–448.

Bhatnagar, A., 2006. Environmental cardiology: studying mechanistic links between pollution and heart disease. Circ. Res. 99, 692–705.

Boland, S., Baeza-Squiban, A., Fournier, T., Houcine, O., Gendron, M.C., Chevrier, M., Jouvenot, G., Coste, A., Aubier, M., Marano, F., 1999. Diesel exhaust particles are taken up by human airway epithelial cells in vitro and alter cytokine production. Am. J. Physiol. 276, L604–L613.

Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S., Silverman, F., 2002. Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105, 1534–1536.

Brook, R.D., Franklin, B., Cascio, W., Hong, Y.L., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Tager, I., 2004. Air pollution and cardiovascular disease—a statement for healthcare professionals from the expert panel on population and prevention science of the American Heart Association. Circulation 109, 2655–2671.

Chang, C.C., Hwang, J.S., Chan, C.C., Wang, P.Y., Cheng, T.J., 2007. Effects of concentrated ambient particles on heart rate, blood pressure, and cardiac contractility in spontaneously hypertensive rats during a dust storm event. Inhal. Toxicol. 19, 973–978.

Chang, C.C., Hwang, J.S., Chan, C.C., Wang, P.Y., Hu, T.H., Cheng, T.J., 2004. Effects of concentrated ambient particles on heart rate, blood pressure, and cardiac contractility in spontaneously hypertensive rats. Inhal. Toxicol. 16, 421–429.

Chen, J., Tan, M., Nemmar, A., Song, W., Dong, M., Zhang, G., Li, Y., 2006. Quantification of extrapulmonary translocation of intratracheal-instilled particles in vivo in rats: effect of lipopolysaccharide. Toxicology 222, 195–201.

Delfino, R.J., Sioutas, C., Malik, S., 2005. Potential role of ultrafine particles in associations between airborne particle mass and cardiovascular health. Environ. Health Perspect. 113, 934–946.

Dubowsky, S.D., Suh, H., Schwartz, J., Coull, B.A., Gold, D.R., 2006. Diabetes, obesity, and hypertension may enhance associations between air pollution and markers of systemic inflammation. Environ. Health Perspect. 114, 992–998.

Elder, A., Gelein, R., Silva, V., Feikert, T., Opanashuk, L., Carter, J., Potter, R., Maynard, A., Ito, Y., Finkelstein, J., Oberdorster, G., 2006. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ. Health Perspect. 114, 1172–1178.

Fielding, C.A., McLoughlin, R.M., McLeod, L., Colmont, C.S., Najdovska, M., Grail, D., Ernst, M., Jones, S.A., Topley, N., Jenkins, B.J., 2008. IL-6 regulates neutrophil trafficking during acute inflammation via STAT3. J. Immunol. 181, 2189–2195.

Geiser, M., Rothen-Rutishauser, B., Kapp, N., Schurch, S., Kreyling, W., Schulz, H., Semmler, M., Hof, V.I., Heyder, J., Gehr, P., 2005. Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Environ. Health Perspect. 113, 1555–1560.

Ghio, A.J., Jaskot, R.H., Hatch, G.E., 1994. Lung injury after silica instillation is associated with an accumulation of iron in rats. Am. J. Physiol 267, L686–L692.

Gold, D.R., Litonjua, A., Schwartz, J., Lovett, E., Larson, A., Nearing, B., Allen, G., Verrier, M., Cherry, R., Verrier, R., 2000. Ambient pollution and heart rate variability. Circulation 101, 1267–1273.

Gurgueira, S.A., Lawrence, J., Coull, B., Murthy, G.G., Gonzalez-Flecha, B., 2002. Rapid increases in the steady-state concentration of reactive oxygen species in the lungs and heart after particulate air pollution inhalation. Environ. Health Perspect. 110, 749–755.

Hicks, A., Monkarsh, S.P., Hoffman, A.F., Goodnow Jr., R., 2007. Leukotriene B4 receptor antagonists as therapeutics for inflammatory disease: preclinical and clinical developments. Expert. Opin. Investig. Drugs 16, 1909–1920.

Kapp, N., Kreyling, W., Schulz, H., Hof, V.I., Gehr, P., Semmler, M., Geiser, M., 2004. Electron energy loss spectroscopy for analysis of inhaled ultrafine particles in rat lungs. Microsc. Res. Tech. 63, 298–305.

Kasperska-Zajac, A., Rogala, B., 2006. Platelet function in anaphylaxis. J. Investig. Allergol. Clin. Immunol. 16, 1–4.

Kato, T., Yashiro, T., Murata, Y., Herbert, D.C., Oshikawa, K., Bando, M., Ohno, S., Sugiyama, Y., 2003. Evidence that exogenous substances can be phagocytized by alveolar epithelial cells and transported into blood capillaries. Cell. Tissue Res. 311, 47–51.

Khandoga, A., Stampfl, A., Takenaka, S., Schulz, H., Radykewicz, R., Kreyling, W., Krombach, F., 2004. Ultrafine particles exert prothrombotic but not inflammatory

effects on the hepatic microcirculation in healthy mice in vivo. Circulation 109, 1320–1325.
Kihara, H., Koganei, H., Hirose, K., Yamamoto, H., Yoshimoto, R., 2001. Antithrombotic activity of AT-1015, a potent 5-HT(2A) receptor antagonist, in rat arterial thrombosis model and its effect on bleeding time. Eur. J. Pharmacol. 433, 157–162.
Kodavanti, U.P., Schladweiler, M.C., Ledbetter, A.D., Hauser, R., Christiani, D.C., Mcgee, J., Richards, J.R., Costa, D.L., 2002. Temporal association between pulmonary and systemic effects of particulate matter in healthy and cardiovascular compromised rats. J. Toxicol. Environ. Health A 65, 1545–1569.
Kunzli, N., Jerrett, M., Mack, W.J., Beckerman, B., LaBree, L., Gilliland, F., Thomas, D., Peters, J., Hodis, H.N., 2005. Ambient air pollution and atherosclerosis in Los Angeles. Environ. Health Perspect. 113, 201–206.
Lee, D.L., Leite, R., Fleming, C., Pollock, J.S., Webb, R.C., Brands, M.W., 2004. Hypertensive response to acute stress is attenuated in interleukin-6 knockout mice. Hypertension 44, 259–263.
Martin, T.R., Pistorese, B.P., Chi, E.Y., Goodman, R.B., Matthay, M.A., 1989. Effects of leukotriene B4 in the human lung. Recruitment of neutrophils into the alveolar spaces without a change in protein permeability. J. Clin. Invest. 84, 1609–1619.
Mills, N.L., Donaldson, K., Hadoke, P.W., Boon, N.A., MacNee, W., Cassee, F.R., Sandstrom, T., Blomberg, A., Newby, D.E., 2009. Adverse cardiovascular effects of air pollution. Nat. Clin. Pract. Cardiovasc. Med. 6, 36–44.
Mills, N.L., Tornqvist, H., Robinson, S.D., Gonzalez, M., Darnley, K., MacNee, W., Boon, N.A., Donaldson, K., Blomberg, A., Sandstrom, T., Newby, D.E., 2005. Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. Circulation 112, 3930–3936.
Mutlu, G.M., Green, D., Bellmeyer, A., Baker, C.M., Burgess, Z., Rajamannan, N., Christman, J.W., Foiles, N., Kamp, D.W., Ghio, A.J., Chandel, N.S., Dean, D.A., Sznajder, J.I., Budinger, G.R., 2007. Ambient particulate matter accelerates coagulation via an IL-6-dependent pathway. J. Clin. Invest. 117, 2952–2961.
Nemmar, A., Al Maskari, S., Ali, B.H., Al Amri, I.S., 2007a. Cardiovascular and lung inflammatory effects induced by systemically administered diesel exhaust particles in rats. Am. J. Physiol. Lung. Cell Mol. Physiol. 292, L664–L670.
Nemmar, A., Hoet, P.H., Dinsdale, D., Vermylen, J., Hoylaerts, M.F., Nemery, B., 2003a. Diesel exhaust particles in lung acutely enhance experimental peripheral thrombosis. Circulation 107, 1202–1208.
Nemmar, A., Hoet, P.H., Vandervoort, P., Dinsdale, D., Nemery, B., Hoylaerts, M.F., 2007b. Enhanced peripheral thrombogenicity after lung inflammation is mediated by platelet-leukocyte activation: role of P-selectin. J. Thromb. Haemost..
Nemmar, A., Hoet, P.H., Vanquickenborne, B., Dinsdale, D., Thomeer, M., Hoylaerts, M.F., Vanbilloen, H., Mortelmans, L., Nemery, B., 2002a. Passage of inhaled particles into the blood circulation in humans. Circulation 105, 411–414.
Nemmar, A., Hoet, P.H.M., Vermylen, J., Nemery, B., Hoylaerts, M.F., 2004a. Pharmacological stabilization of mast cells abrogates late thrombotic events induced by diesel exhaust particles in hamsters. Circulation 110, 1670–1677.
Nemmar, A., Hoylaerts, M.F., Hoet, P.H., Nemery, B., 2004b. Possible mechanisms of the cardiovascular effects of inhaled particles: systemic translocation and prothrombotic effects. Toxicol. Lett. 149, 243–253.
Nemmar, A., Hoylaerts, M.F., Hoet, P.H.M., Dinsdale, D., Smith, T., Xu, H., Vermylen, J., Nemery, B., 2002b. Ultrafine particles affect experimental thrombosis in an *in vivo* hamster model. Am. J. Respir. Crit. Care Med. 166, 998–1004.
Nemmar, A., Melghit, K., Ali, B.H., 2008. The acute proinflammatory and prothrombotic effects of pulmonary exposure to rutile TiO_2 nanorods in rats. Exp. Biol. Med. (Maywood.) 233, 610–619.
Nemmar, A., Nemery, B., Hoet, P.H.M., Van Rooijen, N., Hoylaerts, M.F., 2005. Silica particles enhance peripheral thrombosis—key role of lung macrophage-neutrophil cross-talk. Am. J. Respir. Crit Care Med. 171, 872–879.
Nemmar, A., Nemery, B., Hoet, P.H.M., Vermylen, J., Hoylaerts, M.F., 2003b. Pulmonary inflammation and thrombogenicity caused by diesel particles in hamsters - Role of histamine. Am. J. Respir. Crit Care Med. 168, 1366–1372.
Nemmar, A., Vanbilloen, H., Hoylaerts, M.F., Hoet, P.H., Verbruggen, A., Nemery, B., 2001. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. Am. J. Respir. Crit Care Med. 164, 1665–1668.
Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., Cox, C., 2004. Translocation of inhaled ultrafine particles to the brain. Inhal. Toxicol. 16, 437–445.
Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Lunts, A., Kreyling, W., Cox, C., 2002. Extrapulmonary translocation of ultrafine carbon particle following whole-body inhalation exposure of rats. J. Toxicol. Environ. Health A 65, 1531–1543.
Pekkanen, J., Peters, A., Hoek, G., Tiittanen, P., Brunekreef, B., de Hartog, J., Heinrich, J., Ibald-Mulli, A., Kreyling, W.G., Lanki, T., Timonen, K.L., Vanninen, E., 2002. Particulate air pollution and risk of ST-segment depression during repeated submaximal exercise tests among subjects with coronary heart disease: the exposure and risk assessment for fine and ultrafine particles in ambient air (ULTRA) study. Circulation 106, 933–938.
Peretz, A., Sullivan, J.H., Leotta, D.F., Trenga, C.A., Sands, F.N., Allen, J., Carlsten, C., Wilkinson, C.W., Gill, E.A., Kaufman, J.D., 2008. Diesel exhaust inhalation elicits acute vasoconstriction in vivo. Environ. Health Perspect. 116, 937–942.
Peters, A., Dockery, D.W., Muller, J.E., Mittleman, M.A., 2001. Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103, 2810–2815.
Rahman, I., Biswas, S.K., Kode, A., 2006. Oxidant and antioxidant balance in the airways and airway diseases. Eur. J. Pharmacol. 533, 222–239.
Riedl, M., Diaz-Sanchez, D., 2005. Biology of diesel exhaust effects on respiratory function. J. Allergy Clin. Immunol. 115, 221–228.
Rodriguez Ferreira Rivero, D.H., Sassaki, C., Lorenzi-Filho, G., Nascimento Saldiva, P.H., 2005. PM(2.5) induces acute electrocardiographic alterations in healthy rats. Environ. Res. 99, 262–266.
Ruckerl, R., Ibald-Mulli, A., Koenig, W., Schneider, A., Woelke, G., Cyrys, J., Heinrich, J., Marder, V., Frampton, M., Wichmann, H.E., Peters, A., 2006. Air pollution and markers of inflammation and coagulation in patients with coronary heart disease. Am. J. Respir. Crit. Care Med. 173, 432–441.
Salvi, S., Blomberg, A., Rudell, B., Kelly, F., Sandstrom, T., Holgate, S.T., Frew, A., 1999. Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. Am. J. Respir. Crit. Care Med. 159, 702–709.
Salvi, S.S., Nordenhall, C., Blomberg, A., Rudell, B., Pourazar, J., Kelly, F.J., Wilson, S., Sandstrom, T., Holgate, S.T., Frew, A.J., 2000. Acute exposure to diesel exhaust increases IL-8 and GRO-alpha production in healthy human airways. Am. J. Respir. Crit. Care Med. 161, 550–557.
Shimada, A., Kawamura, N., Okajima, M., Kaewamatawong, T., Inoue, H., Morita, T., 2006. Translocation pathway of the intratracheally instilled ultrafine particles from the lung into the blood circulation in the mouse. Toxicol. Pathol. 34, 949–957.
Silva, V.M., Corson, N., Elder, A., Oberdorster, G., 2005. The rat ear vein model for investigating in vivo thrombogenicity of ultrafine particles (UFP). Toxicol. Sci. 85, 983–989.
Ulker, O., Yucesoy, B., Demir, O., Tekin, I., Karakaya, A., 2008. Serum and BAL cytokine and antioxidant enzyme levels at different stages of pneumoconiosis in coal workers. Hum. Exp. Toxicol. 27, 871–877.
Urch, B., Silverman, F., Corey, P., Brook, J.R., Lukic, K.Z., Rajagopalan, S., Brook, R.D., 2005. Acute blood pressure responses in healthy adults during controlled air pollution exposures. Environ. Health Perspect. 113, 1052–1055.
Valenca, S.S., Silva, B.F., Lopes, A.A., Romana-Souza, B., Marinho Cavalcante, M.C., Lima, A.B., Goncalves, K.V., Porto, L.C., 2008. Oxidative stress in mouse plasma and lungs induced by cigarette smoke and lipopolysaccharide. Environ. Res. 108, 199–204.
Vermylen, J., Nemmar, A., Nemery, B., Hoylaerts, M.F., 2005. Ambient air pollution and acute myocardial infarction. J. Thromb. Haemost. 3, 1955–1961.
Welsh, P., Woodward, M., Rumley, A., Lowe, G., 2008. Associations of plasma pro-inflammatory cytokines, fibrinogen, viscosity and C-reactive protein with cardiovascular risk factors and social deprivation: the fourth Glasgow MONICA study. Br. J. Haematol. 141, 852–861.
Zappulla, D., 2008. Environmental stress, erythrocyte dysfunctions, inflammation, and the metabolic syndrome: adaptations to CO2 increases? J. Cardiometab. Syndr. 3, 30–34.
Zarbock, A., Singbartl, K., Ley, K., 2006. Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. J. Clin. Invest. 116, 3211–3219.