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Original Paper

Short-Term Systemic Effects of Nose-Only Cigarette Smoke Exposure in Mice: Role of Oxidative Stress

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Key Words
Cigarette smoke • Nose-only exposure, short-term exposure • Systolic blood pressure • Thrombosis • Liver enzymes • Oxidative stress

Abstract
Background/Aims: Long–term cigarette smoking (CS) is a major risk factor for respiratory and cardiovascular diseases, and is also known to adversely affect other organs. However, data on the systemic effects of short-term CS exposure (STCSE) are scarce. Presently, using a nose-only exposure system, we evaluated the systemic effects of STCSE in mice. ***Methods:*** We assessed the effects of CS generated by 9 consecutive cigarettes per day for 4 days in a nose-only exposure system on cardiovascular, hepatic and renal endpoints evaluated on day 5 in mice. Control mice were exposed to air only. ***Results:*** CS significantly increased systolic blood pressure and decreased total nitric oxide plasma concentration. Circulating platelets and erythrocyte numbers were also increased. However, STCSE did not significantly increase thrombosis in pial arterioles and venules. STCSE significantly raised plasma alanine aminotransferase and gamma glutamyl transpeptidase activities, but did not affect urea or creatinine concentrations. Interestingly, while STCSE enhanced the production of reactive oxygen species in heart and kidney and lipid peroxidation in heart, liver and kidneys, it also enhanced the antioxidant activity of superoxide dismutase, probably indicating that STCSE causes adaptive reactions to counterbalance the potentially damaging action of oxygen radicals induced by STCSE. ***Conclusion:*** These results suggest that STCSE causes blood pressure increase, hepatotoxicity and oxidative stress in the heart, liver and the kidneys. These data provide information on the initial steps leading to the systemic effects of STCSE, a stage at which the diseases may likely be reversed.

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Introduction

Cigarette smoke (CS) is a major risk factor for chronic obstructive pulmonary disease (COPD), lung cancer, and cardiovascular diseases. COPD is characterized by an excessive inflammatory response in the airways, parenchyma and pulmonary vasculature [1, 2].

In addition to the typical pulmonary changes in COPD, several extrapulmonary effects have been recognized [1, 2]. Several studies have reported the occurrence of systemic events following CS exposure including increase in plasma concentrations of proinflammatory cytokines and oxidative stress [1, 2]. Moreover, it has been recognized that systemic features and other diseases are more common in COPD, including skeletal muscle dysfunction, cardiovascular disease, and diabetes, all of which are thought to have an analogous inflammation-based mechanisms to COPD [1].

Even in the absence of COPD, CS alone can cause significant extrapulmonary diseases such as coronary artery disease. Young smokers or even passive smokers may show endothelial dysfunction of the systemic vessels and systemic oxidative stress [3, 4]. Oxidative stress causes muscle fatigue and facilitates proteolysis [5].

Extensive evidence has been reported on the pulmonary and systemic effects of chronic CS exposure. Such experiments require chronic exposure to CS lasting for several months [1, 6]. However, there is a paucity of data on the short-term effects of CS. The later can give specific information on the initial changes in the pulmonary and extrapulmonary sites, and will likely offer the possibility to reverse them.

We have recently demonstrated that short-term nose-only exposure to CS, a system that best resembles the human exposure situation, induced increase in airway resistance, pulmonary inflammation and morphological changes, and oxidative stress in the lung tissue [7]. Several studies investigated the effect of CS on oxidative stress in various organs including skeletal muscle, lung, liver, kidney or bladder muscle [8-10]. However, as far as we are aware, no study has comprehensively assessed the short-term nose-only effects of CS on cardiovascular system (blood pressure, circulatory cells and thrombosis), liver and kidney functions and the role of oxidative stress thereon. Therefore, the specific aim of this study is to investigate the systemic effects of short-term nose-only CS exposure on systolic blood pressure, pial microvessel thrombosis, liver and kidney functions, and the effect on oxidative stress comprising the concentrations of reactive oxygen species and lipid peroxidation and superoxide dismutase activity in the heart, liver and kidney.

Materials and Methods

Animals and treatments

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 (8/4/2012, protocol No. A7-12,).

CS exposure

BALB/C mice (Taconic Farms Inc., Germantown, NY, USA) were housed in a conventional animal house and maintained on a 12-hour light-dark cycle. The animals were placed in cages and supplied with pelleted food and water *ad libitum*. Following an acclimatization period of one week, animals were randomly divided into two groups: control (air) and CS-exposed. Mice were placed in soft restraints and connected to the exposure tower. Animals were exposed to mainstream CS generated by commercially available filtered cigarettes (Marlboro red, 12 mg tar/1.0 mg nicotine; Philip Morris, Richmond, VA) through their noses using a nose-only exposure system (InExpose System, Scireq, Canada). A computer-controlled puff was generated every minute, leading to 10 s of CS exposure followed by 50 s of fresh air. CS-exposed group inhaled CS from 9 consecutive cigarettes per day for 4 days as previously described [7, 11]. Control animals were treated similarly but were exposed to filtered air for the same duration. The total particulate density concentration

of CS was measured daily, and indicated an average of 420.5 mg total particulate matter per m^3 (TPM/m^3) in the tower [7].

Systolic blood pressure (SBP) measurement

Following the exposure to CS, the systolic BP (SBP) was measured using a computerized noninvasive tail-cuff manometry system (ADInstrument, Colorado Springs, USA). To avoid procedure-induced anxiety, mice were trained for 5 consecutive days before the experimental procedure [12].

Blood collection and analysis

The same animals used to measure SBP were anesthetized with intraperitoneal injection of sodium pentobarbital (45 mg/kg), and blood was drawn from the inferior vena cava in EDTA (4 %). A sample was used for platelets and white blood cells (WBC) counts using an ABX VET ABC Hematology Analyzer with a mouse card (ABX Diagnostics, Montpellier, France). The remaining blood was centrifuged at 4°C for 15 min at 900 *g* and the plasma samples were stored at –80°C until further analysis.

The determination of nitric oxide (NO) was performed with a total NO assay kit from R & D systems (Minneapolis, MN, USA) which measures the more stable NO metabolites NO_2^- and NO_3^-[13, 14].

The alanine aminotransferase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT) activities, and urea and creatinine concentrations were measured using standard laboratory methods with LX20 multiple automated analyser (Beckman Coulter, CA, USA).

Measurement of oxidative stress, lipid peroxidation and superoxide dismutase (SOD) activity in heart, liver and kidneys

Following the exposure to CS or air, animals were sacrificed by an overdose of sodium pentobarbital, and their heart, liver and kidney tissues were quickly collected and rinsed with ice-cold PBS (pH 7.4) before homogenization in 0.1M phosphate buffer pH 7.4 containing 0.15M KCl, 0.1mM EDTA, 1mM DTT and 0.1mM phenylmethylsulfonylfluoride at 4°C. Homogenates were centrifuged for 10 min at 3000xg to remove cellular debris and supernatants were used for further analysis. Protein content was measured by Bradford's method as described before [15, 16].

Measurement of ROS and lipid peroxidation: ROS in heart, liver and kidney tissues of all mice were measured using 2', 7'- Dichlorofluorescein diacetate (DCFDA; Molecular Probes, Eugene, OR, USA) as a fluorescent probe as described before [17, 18]. NADPH-dependent membrane lipid peroxidation was measured as thiobarbituric acid reactive substance using malonedialdehyde as standard (Sigma-Aldrich Fine Chemicals, St Louis, MO, USA) [18].

SOD activity was measured as the conversion of NBT to NBT-diformazan according to the vendor's protocol (R & D System, MN, USA). The extent of reduction in the appearance of NBT-formazan was used as a measure of SOD activity present in the tissues.

Experimental pial arterioles and venules thrombosis model

In a separate experiment, *in vivo* thrombogenesis in the pial arterioles and venules was assessed after CS exposure, according to a previously described technique [19, 20]. Briefly, the trachea was intubated after induction of anaesthesia with urethane (1mg/g body weight, i.p.), and a 2F venous catheter (Portex, Hythe, UK) was inserted in the right jugular vein for the administration of fluorescein (Sigma, St. Louis, MO, USA). After that, a craniotomy was first performed on the left side, using a microdrill, and the dura was stripped open. Only untraumatized preparations were used, and those showing trauma to either microvessels or underlying brain tissue were discarded. The animals were then placed on the stage of a fluorescence microscope (Olympus, Melville, NY, USA) attached to a camera and DVD recorder. A heating mat was placed under the mice and body temperature was raised to 37°C, as monitored by a rectal thermoprobe connected to a temperature reader (Physitemp Instruments, NJ, USA). The cranial preparation was moistened continuously with artificial cerebrospinal fluid of the following composition (mM): NaCl 124, KCl 5, NaH_2PO_4 3, $CaCl_2$ 2.5, $MgSO_4$.4, $NaHCO_3$ 23 and glucose 10, pH 7.3-7.4. A field containing arterioles and venules 15-20 μm in diameter was chosen. Such a field was taped prior to, and during the photochemical insult, which was carried out by injecting fluorescein (0.1ml/mouse of 5% solution) via the jugular vein, which was allowed to circulate for 30-40 sec. The cranial preparation was then exposed to stabilized mercury light. The combination produces endothelium injury of the arterioles. This, in turn, causes platelets to adhere

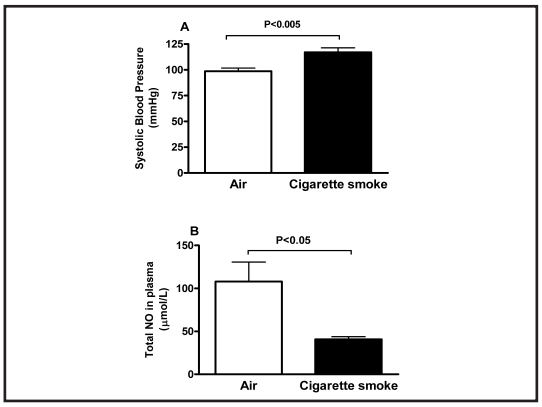

Fig. 1. Systolic blood pressure (A), and total nitric oxide (NO) levels in plasma (B) following short-term nose-only cigarette smoke exposure or air (control). Data are mean ± SEM (n = 8).

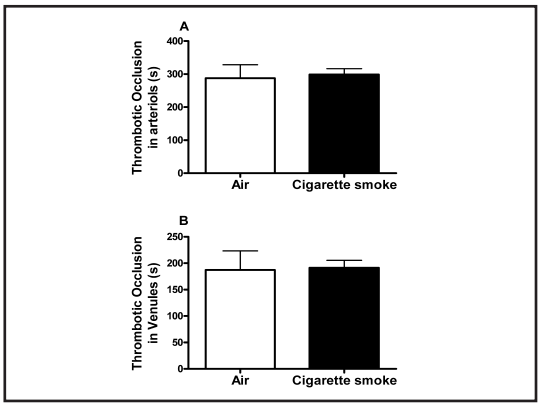

Fig. 3. Thrombotic occlusion time in pial arterioles (A) and venules (B) following short-term nose-only cigarette smoke or air (control) exposure. Data are mean ± SEM (n = 8).

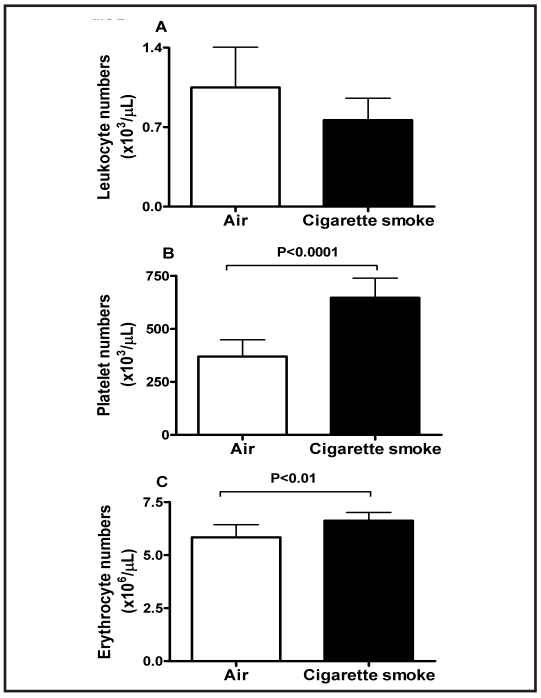

Fig. 2. Leukocyte (A), platelet (B) and erythrocyte (C) numbers following short-term nose-only cigarette smoke or air (control) exposure. Data are mean ± SEM (n = 8).

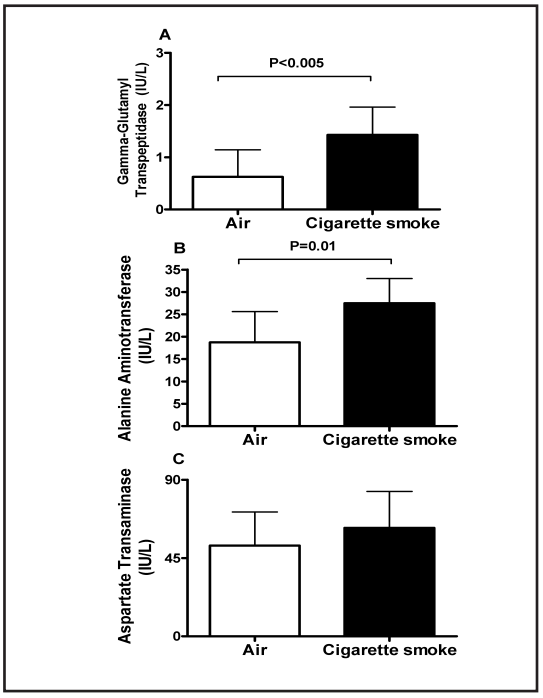

Fig. 4. Gamma-glutamyl transpeptidase (A), alanine aminotranferease (B) and aspartate transaminase (C) activities in plasma following short-term nose-only cigarette smoke or air (control) exposure. Data are mean ± SEM (n = 8).

at the site of endothelial damage and then aggregate. Platelet aggregates and thrombus formation grow in size until complete vascular occlusion. The time from the photochemical injury until full vascular occlusion (time to flow stop) in arterioles were measured in seconds. At the end of the experiments, the animals were euthanized by an overdose of urethane.

Statistics

All statistical analyses were performed with GraphPad Prism Software version 4. (San Diego, CA, USA). To determine whether parameters were normally distributed, the Kolmogorov–Smirnov statistic normality

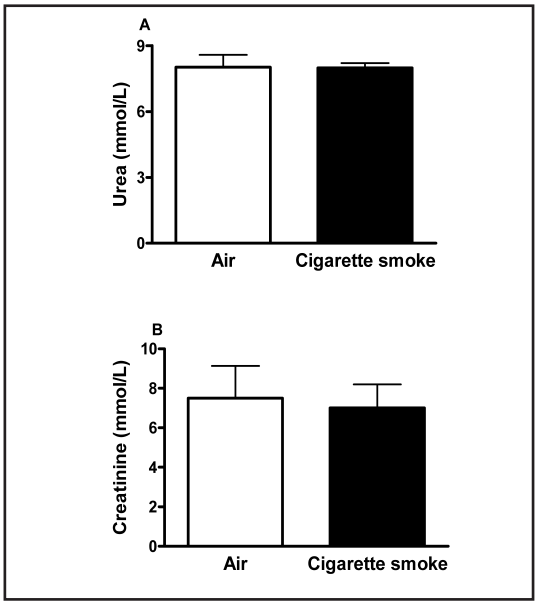

Fig. 5. Urea (A) and creatinine (B) in plasma following short-term nose-only cigarette smoke or air (control) exposure. Data are mean ± SEM (n = 8).

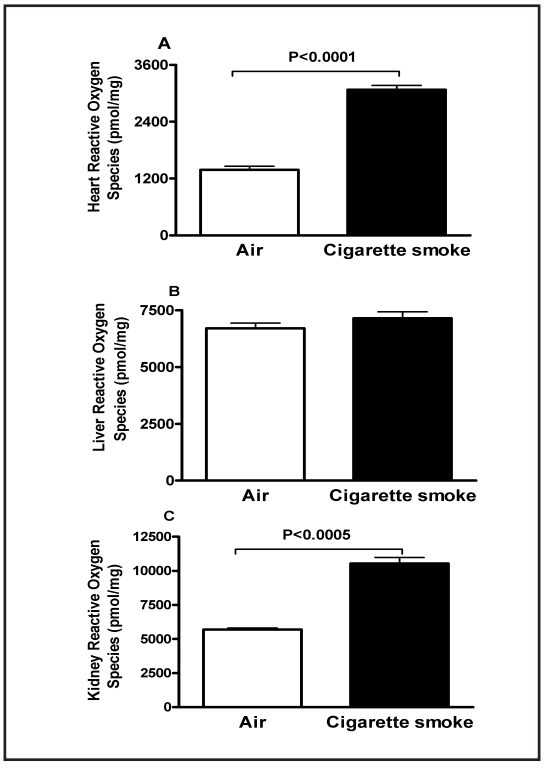

Fig. 6. Reactive oxygen species in the heart (A), liver (B) and kidney (C) tissues following short-term nose-only cigarette smoke or air (control) exposure. Data are mean ± SD (n=8).

test was applied. Normally distributed data were analyzed using the unpaired *t*-test for differences between groups whereas non-normally ones where analyzed with Mann Whitney test. *P*-values <0.05 were considered as significant. All the data in figures were reported as mean ± SEM.

Results

Systolic blood pressure and NO levels in plasma

Short-term exposure to CS caused a significant increase in SBP compared to air-exposed mice (Fig. 1A). The NO (which is known to diffuse into vascular smooth muscle cells and causes vasorelaxation) levels have significantly decreased in plasma of mice exposed to CS compared to air-exposed mice (Fig. 1B).

Leukocyte, platelet and erythrocyte numbers in blood

Compared to control group, short-term nose-only CS exposure caused a significant increase in platelet (P<0.0001) and erythrocyte (P<0.01) numbers (Fig. 2B-C). However, the number of leukocytes was not significantly affected by CS exposure (Fig. 2A).

Thrombosis in pial venules and arterioles

The assessment of thrombosis in pial arterioles and venules revealed the absence of prothrombotic effect of CS at the studied time point. Figure 3 shows that compared to air-exposed mice, the thrombotic occlusion time in arterioles and venules were not affected following short-term exposure to CS.

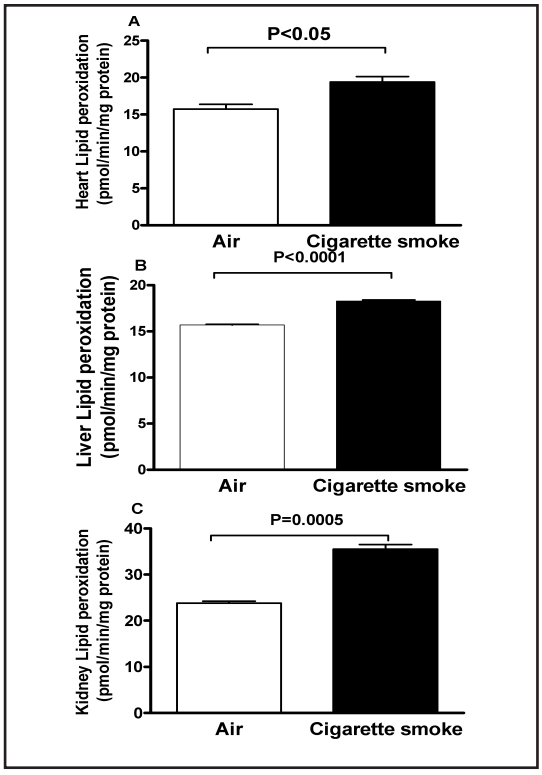

Fig. 7. Lipid peroxidation in the heart (A), liver (B) and kidney (C) tissues following short-term nose-only cigarette smoke or air (control) exposure. Data are mean ± SD (n=8).

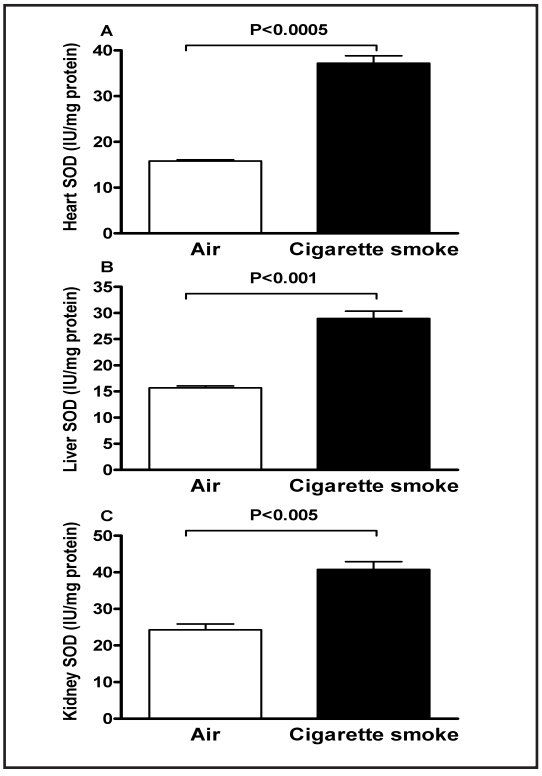

Fig. 8. Superoxide dismutase in the heart (A), liver (B) and kidney (C) tissues following short-term nose-only cigarette smoke or air (control) exposure. Data are mean ± SD (n=8).

GGT, ALT and AST in plasma

Short-term nose-only exposure to CS caused a significant increase in the activity of GGT (P<0.005) and ALT (P=0.01) compared to control group (Fig. 4A-B). The AST activity was slightly but insignificantly increased following short-term exposure to CS.

Urea and Creatinine in plasma

Figure 5 illustrates the effect of CS on plasma concentrations of urea and creatinine. Following short-term nose-only CS exposure, the plasma concentrations of urea and creatinine were not increased significantly compared to air-exposed mice.

*Reactive oxygen species concentrations in heart, liver and kidney tissues*Short-term nose-only CS exposure induced a significant increase in the concentration of reactive oxygen species in heart (P<0.0001) and kidney (P<0.0005) tissues compared to air exposed group (Fig. 6A and C). In the liver, the observed increase of reactive oxygen species concentration did not reach statistical significance (Fig. 6B).

Lipid peroxidation in heart, liver and kidney tissues

The effect of short-term nose-only CS on lipid peroxidation in the heart, liver and kidney is depicted in Figure 7. Compared to control group, a significant increase in lipid peroxidation were observed in the heart (P<0.05; Fig. 7A), liver (P<0.0001; Fig. 7B) and kidney (P=0.0005; Fig. 7C) following short-term nose-only exposure to CS.

SOD activity in the heart, liver and kidney tissues

Figure 8 illustrates the effect of short-term nose-only CS exposure on the concentrations of the antioxidant SOD in heart, liver and kidney tissues. CS exposure caused a significant increase of SOD activity in the heart ($P<0.0005$, Fig. 8A), liver ($P<0.001$, Fig. 8B) and kidney ($P<0.005$, Fig. 8C) tissues compared to their respective control group.

Discussion

The present work provides evidence that short-term nose-only exposure to CS induced an increase in systolic blood, platelet and erythrocyte numbers and tissue-specific enzymes including AST and GGT but without causing thrombotic events in pial venules or arterioles. Moreover, markers of oxidative stress were all increased in the heart, liver and kidney tissues of mice exposed to CS.

While substantial information has been reported on the systemic effects of chronic smoke exposure, there is a paucity of data on the short-term systemic effects of smoking. One of the reasons why it is important to know these effects is because repetitive short-term smoke effects may constitute the underlying causal chain of reactions leading to the ultimate chronic effects. Therefore, studying the short-term effect of CS can give more specific information and reflect the initial changes in the pathophysiological mechanisms of CS-induced chronic systemic effects.

Several exposure systems are being used to study the effect CS exposure in mice [8, 21, 22]. The smoking machines that have been employed with animal models comprise systems that use nose-only or whole body exposures. The shortcoming of using whole body exposure is that the animals may ingest nicotine or tar substances when cleaning their fur. The nose-only exposure system avoids this problem and most likely best resembles the human situation [8, 21, 22]. Using the nose-only exposure system, we have recently demonstrated CS exposure causes an increase in airway resistance, pulmonary inflammation and morphological changes, and oxidative stress in the lung tissue [7]. The advantage of the current study is that we assessed the extrapulmonary effects of short-term nose-only exposure to CS on various set of indices comprising SBP, circulatory cells, pial thrombosis, liver and kidney functions and the oxidative stress in the heart, liver and kidney.

It has been reported that chronic exposure CS exposure can increase systemic oxidative stress [1], alter NO bioavailability [23], cause endothelial dysfunction [24, 25], and influence the levels of other major risk factors, such as blood pressure [26]. Our data show that short-term exposure to CS induces a significant increase in SBP. Along with that, we observed a significant decrease in the concentration of total NO in plasma following short-term exposure to CS. Our data obtained on day 5 post-CS exposure are in line with those of Guo et al. [27] who reported that short-term (6 weeks) and long-term (16 weeks) CS exposure cause increase in arterial pressure and a marked decrease in NO metabolite. They also reported a correlation between NO and the change of structural and mechanical status of arterial wall in response to CS [27].

We found that short-term exposure to CS causes a significant increase in platelet and erythrocyte numbers. This finding is suggestive of a rapid bone marrow response, causing an increase in RBC and platelet numbers. Recently, it was reported that acute exposure to nanoparticles and particulate air pollution caused a rapid elevation in platelet counts [28, 29]. Tamagawa et al. [28] found that the intensity of the bone marrow response correlated with the amount of particles phagocytosed by alveolar macrophages in the lung, indicating a strong link between lung and systemic events. The increase in RBCs that we observed in our study is in line with the finding of Kung et al. [30] who reported an increase of RBC and haematocrit in young men smokers. Furthermore, it is well established that higher red blood cell counts, hematocrit, blood viscosity, and an ongoing inflammatory process potentiate the prothrombotic process associated with smoke exposure [31].

The prothrombotic effects of exposure to CS have been repeatedly demonstrated before [31]. It has been reported that CS exposure causes alterations in platelet function, antithrombotic/prothrombotic factors, and fibrinolytic factors [31]. The lack of prothrombotic effects in pial arterioles and venules observed in our study suggest that short-term exposure to CS does not cause prothrombotic effects. Dong et al. [32] demonstrated that the effects of CS on thrombosis require a proatherosclerotic background. Indeed, they reported that the exposure to CS (4h/day, 5 days/week for 12 weeks) causes prothrombotic effects in the carotid artery of $ApoE^{-/-}$ mice but not in wild-type C57Bl/6 mice. The later displayed similar fibrinogen binding and thrombotic occlusion time similar to air-exposed mice [32].

Our data show a significant increase of GGT and ALT, indicating that CS causes hepatotoxicity. This finding points up that the liver might be a potential target organ for short-term CS toxity. Increases in liver enzymes were reported in young men (age range, 18.6-22.8 yr; mean age, 19.4 yr) exposed to CS and were closely correlated with number of cigarettes smoked daily [30]. The absence of a signifcant effect on urea and creatinine plasma concentration by short-term exposure to CS suggests lack of a gross insult to the kidneys, but does not necessarily rule out an adverse effect on renal function, as it has been shown that the classical kidney function tests such as the urea and creatinine may not detect subtle renal insults [33].

There is strong evidence showing a consistent association between cigarette smoking and increase in the mortality rate from smoking-related diseases such as pulmonary and cardiovascular diseases [2]. The role of free radicals in the pathogenesis of these diseases has been well documented [2, 34]. Formation of free radicals and ROS is a normal consequence of a variety of biochemical reactions. However, these free radicals can cause oxidative damage to the tissues through lipid peroxidation. The human body has defence mechanism, which comprise free radical scavenger enzymes namely superoxide dismutase, catalase and glutathione peroxidase [6, 35]. To further assess the mechanism underlying the extrapulmonary effects of short-term exposure to CS, we have assessed the ROS and lipid peroxidation in heart, liver and kidneys. Our findings show that short-term exposure to CS cause oxidative stress in the heart, liver and kidneys. The mechanisms behind these extrapulmonary effects remain to be investigated but could result from the inflammation and oxidative stress taking place in the lung that result in overspill in circulation causing systemic inflammation and oxidative stress affecting various organs [1]. Moreover, our data show a significant increase of the activity of the antioxidant enzyme SOD in the heart, liver and kidneys. This indicates that the development of oxidative stress is accompanied by an adaptive response that counterbalances the potentially damaging activity of oxygen free radicals by antioxidant defence mechanisms. We and others have reported an increase pulmonary oxidative stress following short-term exposure to CS [7, 36] . It has been shown that immediately following exposure to mainstream CS by nose-only inhalation (at varying doses 40, 120, 240 puffs), dose-dependent decreases in pulmonary and renal GSH were observed in rats whereas, in guinea pigs, reductions in pulmonary, hepatic and renal GSH were observed only at the highest level of exposure [9]. Others reported no effects of nose-only CS exposure on GSH content in muscle or lung homogenates in guinea pig lungs 3h and 24h following nose-only CS exposure [8]. Inhalation of CS for 30 days, three times a day in rats, resulted in a significant decrease of the total free glutathione contents in the lung and liver but not in the heart and kidney [10]. Also, elevated concentrations of oxidized glutathione and protein S-thiolation were observed in the lung but not in other tissues [10]. These discrepancies could be explained by species differences in metabolizing the components of CS, protocol of exposure, duration of exposure or other unknown factors. Further studies are required to clarify this issue.

In conclusion, short-term exposure to CS induced increase in systolic blood pressure, platelet and erythrocyte numbers and liver enzymes including AST and GGT but without causing thrombotic events in pial venules or arterioles. Moreover, CS exposure induces oxidative stress in the heart, liver and kidney tissues of mice. Further work is needed to elucidate the celluar and molecualr mechanims underlying these effects.

Acknowledgements

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References

1 Sinden NJ, Stockley RA: Systemic inflammation and comorbidity in COPD: a result of 'overspill' of inflammatory mediators from the lungs? Review of the evidence. Thorax 2010;65:930-936.
2 MacNee W: Pulmonary and systemic oxidant/antioxidant imbalance in chronic obstructive pulmonary disease. Proc Am Thorac Soc 2005;2:50-60.
3 Celermajer DS, Adams MR, Clarkson P, Robinson J, McCredie R, Donald A, Deanfield JE: Passive smoking and impaired endothelium-dependent arterial dilatation in healthy young adults. N Engl J Med 1996;334:150-154.
4 Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LJ: Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. N Engl J Med 1995;332:1198-1203.
5 Rabinovich RA, Ardite E, Troosters T, Carbo N, Alonso J, Gonzalez de Suso JM, Vilaro J, Barbera JA, Polo MF, Argiles JM, Fernandez-Checa JC, Roca J: Reduced muscle redox capacity after endurance training in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2001;164:1114-1118.
6 van der Vaart, Postma DS, Timens W, Ten Hacken NH: Acute effects of cigarette smoke on inflammation and oxidative stress: a review. Thorax 2004;59:713-721.
7 Nemmar A, Raza H, Subramaniyan D, John A, Elwasila M, Ali BH, Adeghate E: Evaluation of the pulmonary effects of short-term nose-only cigarette smoke exposure in mice. Exp Biol Med (Maywood) 2012.
8 Ardite E, Peinado VI, Rabinovich RA, Fernandez-Checa JC, Roca J, Barbera JA: Systemic effects of cigarette smoke exposure in the guinea pig. Respir Med 2006;100:1186-1194.
9 Bilimoria MH, Ecobichon DJ: Protective antioxidant mechanisms in rat and guinea pig tissues challenged by acute exposure to cigarette smoke. Toxicology 1992;72:131-144.
10 Park EM, Park YM, Gwak YS: Oxidative damage in tissues of rats exposed to cigarette smoke. Free Radic Biol Med 1998;25:79-86.
11 Vlahos R, Bozinovski S, Chan SP, Ivanov S, Linden A, Hamilton JA, Anderson GP: Neutralizing granulocyte/macrophage colony-stimulating factor inhibits cigarette smoke-induced lung inflammation. Am J Respir Crit Care Med 2010;182:34-40.
12 Ying Z, Yue P, Xu X, Zhong M, Sun Q, Mikolaj M, Wang A, Brook RD, Chen LC, Rajagopalan S: Air pollution and cardiac remodeling: a role for RhoA/Rho-kinase. Am J Physiol Heart Circ Physiol 2009;296:H1540-H1550.
13 Wennmalm A, Benthin G, Edlund A, Jungersten L, Kieler-Jensen N, Lundin S, Westfelt UN, Petersson AS, Waagstein F: Metabolism and excretion of nitric oxide in humans. An experimental and clinical study. Circ Res 1993;73:1121-1127.
14 Tsikas D: Methods of quantitative analysis of the nitric oxide metabolites nitrite and nitrate in human biological fluids. Free Radic Res 2005;39:797-815.
15 Raza H, John A, Lakhani MS, Ahmed I, Montague W: Multiplicity and tissue specific expression of camel cytochrome P450(s). Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 1998;121:205-211.
16 Raza H, Ahmed I, John A, Sharma AK: Modulation of xenobiotic metabolism and oxidative stress in chronic streptozotocin-induced diabetic rats fed with Momordica charantia fruit extract. J Biochem Mol Toxicol 2000;14:131-139.
17 Bhagwat SV, Vijayasarathy C, Raza H, Mullick J, Avadhani NG: Preferential effects of nicotine and 4-(N-methyl-N-nitrosamine)-1-(3-pyridyl)-1-butanone on mitochondrial glutathione S-transferase A4-4 induction and increased oxidative stress in the rat brain. Biochem Pharmacol 1998;56:831-839.
18 Raza H, Prabu SK, Robin MA, Avadhani NG: Elevated mitochondrial cytochrome P450 2E1 and glutathione S-transferase A4-4 in streptozotocin-induced diabetic rats: tissue-specific variations and roles in oxidative stress. Diabetes 2004;53:185-194.

19 Nemmar A, Al Salam S, Dhanasekaran S, Sudhadevi M, Ali BH: Pulmonary exposure to diesel exhaust particles promotes cerebral microvessel thrombosis: protective effect of a cysteine prodrug l-2-oxothiazolidine-4-carboxylic acid. Toxicology 2009;263:84-92.
20 Nemmar A, Al-Salam S, Zia S, Marzouqi F, Al-Dhaheri A, Subramaniyan D, Dhanasekaran S, Yasin J, Ali BH, Kazzam EE: Contrasting actions of diesel exhaust particles on the pulmonary and cardiovascular systems and the effects of thymoquinone. Br J Pharmacol 2011;164:1871-1882.
21 Rinaldi M, Maes K, De Vleeschauwer S., Thomas D, Verbeken EK, Decramer M, Janssens W, Gayan-Ramirez GN: Long-term nose-only cigarette smoke exposure induces emphysema and mild skeletal muscle dysfunction in mice. Dis Model Mech 2012;5:333-341.
22 Barreiro E, Peinado VI, Galdiz JB, Ferrer E, Mann-Corral J, Sanchez F, Gea J, Barbera JA: Cigarette Smoke-induced Oxidative Stress A Role in Chronic Obstructive Pulmonary Disease Skeletal Muscle Dysfunction. Am J Respir Crit Care Med 2010;182:477-488.
23 Zhang WZ, Venardos K, Chin-Dusting J, Kaye DM: Adverse effects of cigarette smoke on NO bioavailability: role of arginine metabolism and oxidative stress. Hypertension 2006;48:278-285.
24 Rahman MM, Laher I: Structural and functional alteration of blood vessels caused by cigarette smoking: an overview of molecular mechanisms. Curr Vasc Pharmacol 2007;5:276-292.
25 Rahman MM, Elmi S, Chang TK, Bai N, Sallam NA, Lemos VS, Moien-Afshari F, Laher I: Increased vascular contractility in isolated vessels from cigarette smoking rats is mediated by basal endothelin release. Vascul Pharmacol 2007;46:35-42.
26 Nakamura K, Barzi F, Lam TH, Huxley R, Feigin VL, Ueshima H, Woo J, Gu D, Ohkubo T, Lawes CM, Suh I, Woodward M: Cigarette smoking, systolic blood pressure, and cardiovascular diseases in the Asia-Pacific region. Stroke 2008;39:1694-1702.
27 Guo X, Oldham MJ, Kleinman MT, Phalen RF, Kassab GS: Effect of cigarette smoking on nitric oxide, structural, and mechanical properties of mouse arteries. Am J Physiol Heart Circ Physiol 2006;291:H2354-H2361.
28 Tamagawa E, Bai N, Morimoto K, Gray C, Mui T, Yatera K, Zhang X, Xing L, Li Y, Laher I, Sin DD, Man SF, van Eeden SF: Particulate matter exposure induces persistent lung inflammation and endothelial dysfunction. Am J Physiol Lung Cell Mol Physiol 2008;295:L79-L85.
29 Nemmar A, Melghit K, Al-Salam S, Zia S, Dhanasekaran S, Attoub S, Al-Amri I, Ali BH: Acute respiratory and systemic toxicity of pulmonary exposure to rutile Fe-doped TiO(2) nanorods. Toxicology 2011;279:167-175.
30 Kung CM, Wang HL, Tseng ZL: Cigarette smoking exacerbates health problems in young men. Clin Invest Med 2008;31:E138-E149.
31 Ambrose JA, Barua RS: The pathophysiology of cigarette smoking and cardiovascular disease: an update. J Am Coll Cardiol 2004;43:1731-1737.
32 Dong A, Caicedo J, Han SG, Mueller P, Saha S, Smyth SS, Gairola CG: Enhanced platelet reactivity and thrombosis in Apoe$^{-/-}$ mice exposed to cigarette smoke is attenuated by P2Y12 antagonism. Thromb Res 2010;126:e312-e317.
33 Fuchs TC, Hewitt P: Biomarkers for drug-induced renal damage and nephrotoxicity-an overview for applied toxicology. AAPS J 2011;13:615-631.
34 MacNee W: Oxidants and COPD. Curr Drug Targets Inflamm Allergy 2005;4:627-641.
35 Yao H, Rahman I: Current concepts on oxidative/carbonyl stress, inflammation and epigenetics in pathogenesis of chronic obstructive pulmonary disease. Toxicol Appl Pharmacol 2011;254:72-85.
36 Valenca SS, Silva BF, Lopes AA, Romana-Souza B, Marinho Cavalcante MC, Lima AB, Goncalves K, V, Porto LC: Oxidative stress in mouse plasma and lungs induced by cigarette smoke and lipopolysaccharide. Environ Res 2008;108:199-204.