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The exposure of male newborn mice to the environmental pollutant 1,2-naphthoquinone results in altered vasomotricity of the pulmonary artery as assessed during adulthood

Abstract

We have previously shown that newborn male (but not female) mice exposed to the air pollutant 1,2-naphthoquinone (1,2-NQ), a compound usually adsorbed on fine particulate matter such as diesel exhaust particles, show an exacerbated lung allergic response at youth. In the present study, we investigated the consequences of the early exposure of mice to 1,2-NQ on the *in vitro* vasomotor activity of pulmonary artery.

Vessels were isolated and mounted in a wire-myograph system in order to evaluate the vasomotor response (contractility and vasorelaxation) to different agents. Activities of the antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione (G) peroxidase (GPx), reductase (GR) and transferase (GST), and nitrotyrosine content in pulmonary arteries and serum samples from both groups were also measured.

The exposure of male mice to 1,2-NQ during the neonatal period resulted in impairment of the *in vitro* pulmonary artery function, in terms of both contraction (induced by phenylephrine and compound U-46619) and relaxation (induced by acetylcholine, sodium nitroprusside, isoproterenol and hydrogen peroxide). In addition, reduced SOD and catalase activities were measured in the vessels in comparison with the control animals.

We thus conclude that, parallel to diminished antioxidant enzyme defences, the exposure of male newborn mice to the pollutant 1,2-NQ results in impairment of pulmonary artery vasomotricity during puberty, thus suggesting that increased cardiovascular risk may occur upon the early exposure to the air pollutant.

Keywords: pulmonary artery, 1,2-naphthoquinone, vascular reactivity, oxidative stress, mice

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Introduction

It is well established that exposure to urban air pollution, including particulate matter, is associated with premature deaths,[1,2] ischemic heart disease,[3–6] stroke,[7,8] chronic obstructive pulmonary disease,[9] acute lower respiratory infections[10] and lung cancer.[1,2] A number of studies have pointed out that vulnerable individuals (e.g. infants and asthmatic patients) and patients with cardiovascular diseases are more susceptible to suffer the above-mentioned conditions.[11–13]

In this regard, particulate matter of variable size and/or composition comprises a complex mixture of suspended solid particles with adsorbed liquid and gaseous compounds. Diesel exhaust particles (DEP) are rich in small sized particulate matter (≤ 2.5 μm), which may penetrate deeply into the airways. These particles can directly translocate to the bloodstream or even induce the release of pro-inflammatory mediators from the lung into circulation.[14,15] In addition, these particles have the ability to bind proteins and serve as carriers of allergens and several organic compounds, which can, in turn, lead to, or exacerbate, a local inflammatory process.[16]

Cho and colleagues[17] have shown that the organic electrophile 1,2-naphthoquinone (1,2-NQ) is present in DEP and ambient air at significant concentrations. In fact, and as previously described, quinone toxicity is based on their ability to cause oxidative stress and modify essential macromolecules, which may be responsible for the biological effects of DEP during allergic diseases.[18–21]

We have recently shown that the exposure of mice to 1,2-NQ during the neonatal phase results in exacerbation of the antigenic response induced with ovalbumin during adulthood, with no significant eosinophilia in the airways or bone marrow, but with moderate leukocyte infiltration into the lungs and significantly increased production of IL-13, TNF-α and LTB_4.[21] We have also observed that the increased susceptibility to allergic lung inflammation secondary to 1,2-NQ exposure does not develop in female mice, which is paralleled by the up-regulation of pulmonary antioxidant defences (unpublished data), thus evidencing gender-related susceptibility to the effects of 1,2-NQ. In fact, special attention has been lately given to gender differences, which were taken into account in studies on pulmonary pathophysiology.[22–24]

In addition, the vascular system is also affected during lung inflammation and oxidative stress occurs in response to air pollutants. Ayer and colleagues[25] observed that the decreased lung volume due to impaired pulmonary function in 8-year-old children exposed to environmental tobacco smoke is associated with increased carotid stiffness. On the other hand, the acute exposure of healthy volunteers to DEP resulted in vascular dysfunction, as assessed by measurement of forearm blood flow.[26] In rats, the exposition to $PM_{2.5}$ during 2 weeks resulted in significant oxidative stress in the pulmonary artery and impairment of the endothelium-dependent relaxation *in vitro*.[27] It is thus clear that the alterations induced by pollutants can target cardiovascular functions, which are probably related to

either an inflammatory response,[5,28,29] or an impaired stimulation of the autonomic nervous system, as a consequence of the activation of afferent nerve endings in the lung.[30]

In this way, the objective of this study was to evaluate the effects of the exposure of male newborn mice to 1,2-NQ on the *in vitro* vasomotor response of pulmonary artery evaluated during at the young adulthood, as well as the consequences on vascular redox status and oxidative modifications.

Material and methods

Drugs and reagents

Isoflurane was obtained from Cristália (Itapira, SP, Brazil) and hydrogen peroxide was from Vetec (Duque de Caxias, RJ, Brazil). Potassium chloride (KCl), acetylcholine (ACh), isoproterenol (Iso), phenylephrine (Phe), sodium nitroprusside (SNP), 1,2 naphthoquinone (1,2-NQ), N_{ω}-Nitro-L-arginine methyl ester hydrochloride (L-NAME) and 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$ (U-46619) were obtained from Sigma Aldrich (St. Louis, MO, USA).

A 100 nM 1,2-NQ solution was freshly prepared in PBS solution (containing 0.001% DMSO and 0.001% Tween 80) under sonication immediately prior to use.

Experimental design

All the experimental protocols were approved by the local ethics committee (CEUA-ICB / USP; protocol Nº 007/2013, page 2, book 3) and performed in accordance with the Brazilian Council for Control of Animal Experimentation (CONCEA) and the European Union Directive 2010/63/EU guidelines.

Male neonate C57BL/6 mice were bred and maintained at our local animal facilities. Mothers remained with the pups at all times, except during the sessions of exposure to the pollutant, until their weaning on day 26. Offsprings were separated by gender and kept in polycarbonate cages (5 animals/cage) in a quiet room with controlled temperature (22±1°C), humidity (65-75%) and a 12 h light–dark cycle, and received standard chow and filtered tap water *ad libitum*. Newborn mice were monitored daily for clinical signs of morbidity (e.g., unusual posture, gait abnormalities, convulsions, tremors or any unusual behaviour such as writhing or self-mutilation) or mortality.

A total of 60 animals were used. They were randomly allocated to one of the two experimental groups (i.e., vehicle or 1,2-NQ). On days 6, 8 and 10 after birth, animals belonging to the same experimental group were housed unrestrained in a polycarbonate cage (surface area 600 cm^2; 5 animals/cage). Using a standard ultrasonic nebulizer (Respiramax; São Paulo, SP, Brazil) directly coupled to the cage, the animals were nebulised during 15 min with either 1,2-NQ vehicle or 100 nM 1,2-NQ solution (thus resulting in a 1,2-NQ-atmosphere environment equivalent to 10 $\mu g/m^3$). On day 43, after an 8 h fasting period, the mice were anesthetized with inhaled isoflurane (3% v/v in O_2) and blood samples were collected using heparin as anticoagulant for preparation of plasma. The animals were then euthanized by exsanguination. The thoracic aorta was excised together with the heart and lungs, and transferred to a cold Krebs solution for immediate isolation of the pulmonary artery.

Isometric tension measurement of pulmonary artery

Pulmonary arteries (PA; 500–600 µm internal diameter) were isolated and at all times kept in a modified Krebs-Ringer solution containing (in mM) 115 NaCl, 25 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$-$7H_2O$, 2.5 $CaCl_2$-$2H_2O$, 0.01 EDTA and 11.1 dextrose, continuously aerated with 95% O_2 plus 5% CO_2 (v/v). After removal of the connective tissue, the arteries were cut into 2 mm segments and mounted on a wire myograph chamber (DMT620; Danish Myo Technology, Denmark) for isometric tension recording (PowerLab; ADInstruments, Colorado Springs, CO). Resting tension was calculated using the normalization module of the software Labchart (version 7; ADInstruments, Colorado Springs, CO) by setting the targeting pressure at 25 mmHg. After a 60 min equilibration period, PA rings were exposed to 60 mM KCl to verify their viability and maximum contraction, and to 1 µM Phe followed by 10 µM ACh in order to check for endothelium integrity. The active tension induced by the agonist was normalized to the maximum tension generated by 60 mM KCl. After the normalization procedure and checking of the vessel integrity, cumulative concentration-response curves were performed for Phe (0.1 nM - 10 µM) and the thromboxane A_2 agonist U44169 (0.1 nM - 10 µM). After pre-contraction of the vessels with Phe, concentration-relaxation curves were performed for ACh (10 pM - 300 µM) or the nitric oxide (NO) donor, SNP (0.1 nM - 0.1 µM). The relaxant responses to ISO (0.1nM - 30 µM) or hydrogen peroxide (10 nM - 100 µM) were also studied after pre-contraction of the vessel rings with U46619. In order to study the participation of endogenous NO, in some experiments the vessels were pre-incubated with 100 µM L-NAME during 15 min.

For all the agonists, the individual log-transformed concentration vs. response curves were plotted. Maximal response (E_{max}) and potency (as pD_2 = -log EC_{50}, being EC_{50} the drug concentration necessary to cause 50% of the maximal response) values were calculated using the software GraphPad Prism (version 5.01; GraphPad Software Inc., USA).

Analysis of vascular antioxidant enzyme activities and protein nitrotyrosine contents

PA samples were homogenized with phosphate buffer (50 mM, pH 7.0) and centrifuged (10,000 *g* during 10 min at 4°C). The resulting supernatant was used to determine the enzymatic activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST). Total protein content was determined according to the method described by Bradford.[31]

For SOD activity measurement, a 10 µl aliquot was added to a mix containing 3 mM xanthine, 0.75 mM XTT (2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide and 56.1 mU/mL xanthine oxidase in 50 mM carbonate buffer (pH 10.2) containing 3 mM EDTA, according to Ukeda and colleagues.[32] The specific SOD activity in the samples is reported as units of SOD per mg of protein, where 1 unit of SOD is defined as the amount of enzyme capable of transforming 1 µmol O_2 per min.

Catalase activity was measured in the PA homogenate supernatants by incubating a 30 µl aliquot in the presence of 10 mM H_2O_2 during 2 min at 25°C. After stopping the reaction by the addition of sodium azide, the remaining H_2O_2 is determined by the oxidation reaction of o-dianisidine in the presence of horseradish peroxidase (HRP), according to Fossati et al.[33] For each sample, a blank reaction consisted in inactivating catalase by incubation of 60°C during 2 h. Results were expressed as units of catalase/mg of protein, where 1 unit of catalase is defined as the amount that degrades 1 µmol H_2O_2 per min at 25°C. GPx activity was determined in each PA homogenate supernatant as previously described by Flohé & Günzler.[34]

GR was assayed according to the method described by Carlberg & Mannervik,[35] by measuring the consumption of NADPH as a

cofactor in the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). Results were expressed as units of GR/mg of protein, where 1 unit of GR is defined as the amount that oxidizes 1 µmol NADPH per min.

GST activity was measured according to the method of Habig et al.,[36] using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. Results were expressed as units of GST/mg of protein, where 1 unit of GST is defined as the amount producing 1 µmol of conjugate of GSH with CDNB per min.

The presence of proteins containing 3-nitrotyrosine (NT) residues in the PA homogenates was analyzed by slot blotting, as previously described.[37] Immunoreactivity of the slots was detected using a chemiluminiscence system (G:BOX Chemi, Syngene, UK) and the band intensities were estimated by densitometric analysis (ImageJ software, USA). Results were normalized by the intensity values obtained after staining of the slots with Ponceau S dye and expressed as arbitrary units.

Plasma analysis

Lipid peroxidation was estimated in the plasma samples by quantification of thiobarbituric reactive species (TBARS) contents, according to the method described by Bird & Draper.[38] The reaction of aldehydes with thiobarbituric acid yields a pink adduct chromophore with peak absorbance at 535 nm. Results are expressed as malondialdehyde (MDA) equivalents, as calculated by using a molar absorption coefficient of 1.55 x 10^5 M/cm.

Protein carbonyl content was evaluated by a slot blotting assay according to the method described by Robinson et al.[39] Briefly, after the application of 2.5 µg of total plasma proteins onto a polyvinylidine difluoride (PVDF) membrane, the reaction with 2,4-dinitrophenylhydrazine (DNPH) in 2 N HCl took place during 5 min. After the washing steps, non-specific protein binding sites were blocked with 0.2% casein and the membrane was further incubated overnight at 18°C with a primary anti-DNP antibody (Abcam, UK), followed by 2 h incubation at room temperature with a secondary goat anti rabbit antibody conjugated to HRP. Detection and quantification of the band intensities were performed as detailed above for NT.

Total NO_x^- anion (i.e., nitrite - NO_2^- plus nitrate - NO_3^-) concentrations in the collected plasma samples were determined by the Griess reaction for nitrite anion after the nitrate reductase-catalyzed reduction of nitrate to nitrite, as previously described.[40]

Statistical analysis

Data are expressed as mean ± SEM (n=5 animals per experimental group); differences between the experimental groups were analysed by one-way ANOVA, followed by the Bonferroni test for *post-hoc* comparisons, using the GraphPad Prism software (version 5.0; GraphPad Software Inc., USA). Values of P<0.05 were considered significant.

Results

Vascular reactivity

As shown in Figure 1, the cumulative addition of either Phe or U46619 resulted in concentration-dependent contractions of the PA rings. Arteries exposed to 1,2-NQ- during the neonatal phase showed higher sensitivity to the α_1 adrenergic agonist Phe (lower pD2) in comparison with the controls, with no statistically different E_{max} values (Figure 1A, Table 1). When the vasoconstrictor response was studied using the thromboxane A_2 agonist U46619, no significant differences were observed in terms of E_{max}, although mean pD_2 value for this agonist was slightly, although significantly, higher in the arteries obtained from the 1,2-NQ-exposed mice.

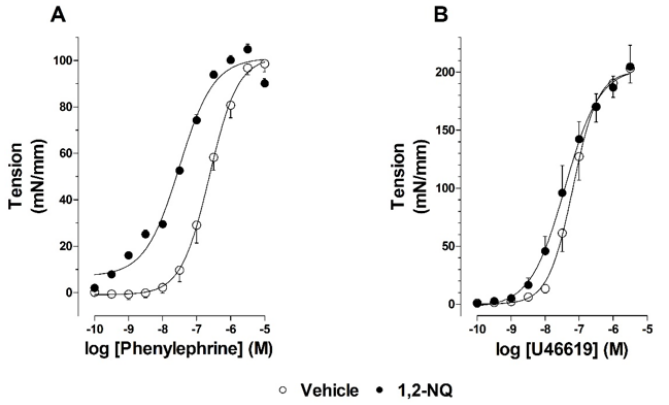

Figure 1 Concentration-response curves to phenylephrine (panel A) and U46619 (panel B) performed in pulmonary arteries rings isolated from young adult male mice exposed to 1,2-NQ or vehicle during the neonatal period. Values are expressed as mean ± SEM; n=5 for all the groups.

In relation to ACh-induced endothelium-dependent relaxation after pre-contraction with Phe, PA rings obtained from mice exposed to 1,2-NQ not only showed lower sensitivity to ACh (higher pD_2 values) but also elevated maximal vasodilatory responses (E_{max}) in comparison with the controls (Figure 2A, Table 1). Blockade of endogenous NO production by L-NAME completely abolished the ACh-induced endothelium-dependent relaxation of the PA rings from all the groups (data not shown), thus evidencing the NO-dependent nature of the ACh response.

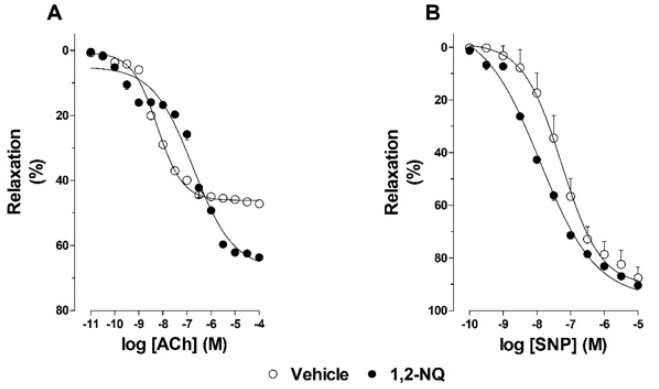

Figure 2 Concentration-response curves to acetylcholine (panel A) and sodium nitroprusside (panel B) performed in phenylephrine-pre-contracted pulmonary arteries rings isolated from young adult male mice exposed to 1,2-NQ or vehicle during the neonatal period. Values are expressed as mean ± SEM; n=5 for all the groups.

Regarding the vasorelaxant responses to the NO donor SNP, PA rings from 1,2-NQ-exposed mice showed lower pD_2 and higher E_{max} values when compared with the vehicle-treated group (Figure 2B, Table 1).

Table 1 E_{max} and pD_2 values (as mean ± SEM; n=5 animals per group) obtained from the concentration-response curves (shown at Figures 1, 2 and 3) performed in pulmonary artery rings obtained from young adult male mice exposed to 1,2-NQ or vehicle during the neonatal period.

	E_{max}		pD_2	
	Control	***1,2-NQ***	***Control***	***1,2-NQ***
Phe	101.5±3.9	101.1±2.3	6.62±0.06	7.51±0.06***
U46619	200.9±8.9	200.4±12.2	7.18±0.07	7.42±0.11**
ACh	46.2±0.4	67.2±1.6***	8.23±0.03	6.74±0.08***
SNP	90.4±2.5	95.2±0.9**	7.26±0.10	7.91±0.06***
Iso	83.1±2.0	80.3±2.0	7.30±0.05	7.29±0.06
H_2O_2	98.6±5.1	87.6±2.1**	4.92±0.05	4.96±0.02

**P<0.01 and ***P<0.001 vs. the vehicle-treated control group.

Table 2 Vascular activities of the antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST), and nitrotyrosine (NT) containing protein contents measured in pulmonary arteries isolated from young adult male mice exposed to 1,2-NQ or vehicle during the neonatal period.

	Control	***1,2-NQ***
SOD (U/mg prot.)	0.35±0.08	0.12±0.01*
Catalase (U/mg prot.)	25.9±5.1	5.0±2.3*
GPx (U/mg prot.)	25.4±3.2	20.4±0.6
GR (U/mg prot.)	7.4±1.7	6.3±0.7
GST (U/mg prot.)	233.1±28.2	149.4±14.5*
NT (A.U.)	0.76±0.14	0.70±0.11

Values are expressed as mean ± SEM; n=5 for all the groups. *P<0.05 vs. the vehicle-treated control group.

Table 3 Total NO_x^- (nitrite + nitrate) anions, protein carbonyl and TBARS concentrations measured in plasma samples collected from young adult male mice exposed to 1,2-NQ or vehicle during the neonatal period.

	Control	***1,2-NQ***
NO_x^- (μM)	23.7±1.5	40.4±4.1*
Protein carbonyl (A.U.)	3.21±0.85	3.22±1.16
TBARS (nmol MDA/ml)	182.2±22.1	160.7±20.3

Values are expressed as mean ± SEM; n=5 for all the groups. *P<0.05 vs. the vehicle-treated control group.

No statistically significant differences due to 1,2-NQ exposure were observed in the vasorelaxant responses of PA rings to isoproterenol (Figure 3A, Table 1). The maximal vasorelaxant response (E_{max}) to hydrogen peroxide was significantly reduced in the PA rings from the 1,2-NQ-exposed mice in comparison with the vehicle-treated group (Figure 3B, Table 1).

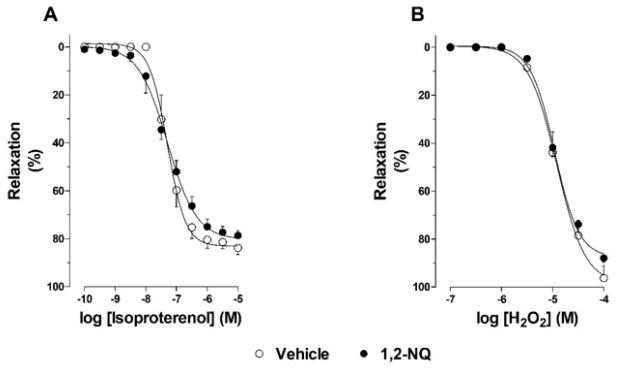

Figure 3 Concentration-response curves to isoproterenol (panel A) and hydrogen peroxide (panel B) performed in U46619-pre-contracted pulmonary arteries rings isolated from young adult male mice exposed to 1,2-NQ or vehicle during the neonatal period. Values are expressed as mean ± SEM; n=4-6 for all the groups.

Vascular antioxidant enzymes and NT-containing proteins

As shown in Table 2, PA obtained from young adult male mice exposed to 1,2-NQ during the neonatal period showed a significant and marked reduction in both SOD and catalase activities as compared to the control animals. Vascular GPx and GR remained unchanged in response to 1,2-NQ exposure. However, GST activity was significantly diminished in the PA obtained from animals exposed to 1,2-NQ during the neonatal period. In addition, significantly higher NT-containing proteins were found in PA from mice secondary to the neonatal exposure to 1,2-NQ.

Plasma analysis

As shown in Table 3, total circulating NO_x^- concentrations were significantly increased in the mice exposed to 1,2-NQ during the newborn period (P<0.05). In contrast, the neonatal exposure of animals to 1,2-NQ had no significant effects on circulating protein carbonyl or TBARS.

Discussion

From a simplistic point of view, pulmonary circulation is a low pressure, low resistance and high flow circuit that accommodates the right ventricle output to the gas exchanging surface in the lung; in this context, pulmonary arteries have a thin smooth muscle layer, which is consistent with a low-pressure system.[41] Endothelial cells of the pulmonary circulation play a central role on vascular tone control by its capacity to release in the vessel milieu several mediators involved in vasoconstriction, vasodilatation, growth and differentiation.[42] It is well known that alterations in the homeostasis of pulmonary artery function may result in the development of several pathological conditions, such as pulmonary hypertension[43] and chronic obstructive pulmonary disease.[9] Indeed, these conditions share the features of being inflammatory processes that gradually evolve at the upper airways with differences between genders.[44]

An early report from Kumagai and colleagues,[45] demonstrated that DEP could inhibit superoxide dismutase activity in the lung, and some years later, the same group showed that 1,2-NQ, among other different quinones present in DEP, was able to supress endothelium-dependent aorta relaxation by directly interacting with eNOS *in vitro*.[46]

However, 1,2-NQ can, not only, induce an acute lung inflammatory response in adult rodents following the administration of a single intra-tracheal dose,[19] but also and more importantly, after weeks after its inhalation, as we have previously demonstrated making use of the protocol described in the present study.[21] The results shown in this study clearly evidence that even 33 days after the exposure of male mice to 1,2-NQ, changes in the vascular reactivity of pulmonary artery are observed at the early adulthood stage. For example, the early exposure to 1,2-NQ resulted in increased potency of Phe, U-46619 and SNP, and higher maximum response to ACh. In addition, considering that L-NAME abolished ACh-induced vasodilatation of the arteries, it is clear that in these vessels, ACh-induced endothelium-dependent vasorelaxation exclusively depends on eNOS-derived NO.

It is also possible that the observed vascular responses secondary to the neonatal exposure to 1,2-NQ are due to some of the already described toxicological characteristics of this compound, such as its alkylating properties, its capability to trigger oxidative stress and the consequent damaging of essential macromolecules.[18,19,21] It has been demonstrated that the exposure to DEP results in activation of a genetic antioxidant response element – ARE (via the transcription factor Nrf2;[47,48] thus leading to upregulation of antioxidant

enzymes (such as heme-oxygenase 1 - HO-1, NAD(P)H-quinone oxidoreductase - NQO1, GST, superoxide dismutase 3 - SOD3 and glucoronosyltransferase[49] with cytoprotective and anti-inflammatory effects.

In this study we also show that the exposure of male mice exposed to 1,2-NQ during the neonatal phase results in significant reduction of SOD, catalase and glutathione S-transferase activities as measured in PA homogenates, in addition to significantly elevated plasma NO_x^- concentrations, in comparison with the control animals. In this way, it is possible that increased amounts of oxygen-derived species (such as superoxide anion, hydrogen peroxide and/or hydroxyl radical) are present under these conditions. Superoxide anion can react with NO to render the highly oxidant peroxynitrite anion - $ONOO^-$, which can, in turn, result not only in oxidative modifications of several biomolecules (such as nitration of protein tyrosine and tryptophan residues, lipids and DNA bases), but also reduce the bioavailability of eNOS-derived NO, and hence lead to the observed impairment of vascular function secondary to endothelial dysfunction.

A deficient clearance of hydrogen peroxide (H_2O_2) due to low catalase / peroxidase activity could facilitate the formation of hydroxyl radical (via Haber-Weiss / Fenton reactions) and the consequent oxidation of different molecular targets which could, in turn, lead to cellular malfunctions. On the other hand, it is well known that H_2O_2 plays a role as a second messenger by activating MAPK cascade[50] and stimulating eNOS for the subsequent NO production.[51] H_2O_2-induced vasodilation has been shown in several vascular beds, including pulmonary artery;[52] in this way, we investigated whether this response could be affected by the neonatal exposure to 1,2-NQ or the consequent effects observed on anti-oxidant enzymes. Curiously, the *in vitro* response of the PA rings to H_2O_2 was very similar between the animal groups, despite the small (non-significant) increase in the maximal vasodilator response to H_2O_2 observed in the male mice PA exposed to 1,2-NQ. It is thus tempting to consider that this slightly altered maximal response could be due to the decreased catalase/GST activity. However, we feel that the physiological relevance of this altered response (as a factor that may contribute to the impairment of endothelium-dependent vasodilation) is questionable, considering that this alteration is only observed at high H_2O_2 concentrations (_0.1 mM), which is very unlikely to occur *in vivo*.

Regarding the increased plasma NO_x^- concentrations observed in the mice exposed to 1,2-NQ, the measurement of systemic NO end-products probably reflects a positive feedback response to the low eNOS-derived NO production mediated by other NOS isoforms.[53]

Conclusion

In conclusion, and in addition to the previously reported lung effects secondary to the neonatal exposure to 1,2-NQ, in the present study we show that pulmonary artery vasomotor activity can also be a target of the long-term 1,2-NQ effects, which, as stated above, may be secondary to redox-mediated mechanisms. As a consequence of the diminished NO bioavailability, the observed vascular alterations in the pulmonary circulation may synergistically worsen the inflammatory process in the lung by facilitating leukocyte-endothelium interaction, increase the production of pro-inflammatory mediators and, consequently, delay the process of resolution of the locally installed inflammatory situation.

In a previously published report, we showed that exposure of neonatal mice to 1,2-NQ results in cardiac adrenergic dysfunction, which may potentially lead to atrial arrhythmias during young adulthood.[54] In this way, the results contained in the present study strengthen the deleterious consequences of exposure to air pollution during the early stages of life by increasing cardiovascular risks in adulthood.

Compliance with ethical standards

All the experimental protocols involving animals were performed in accordance with the Brazilian Council for Control of Animal Experimentation (CONCEA) and the European Union Directive 2010/63/EU guidelines, and were approved by the local ethics committee (CEUA-ICB / USP; protocol Nº 007/2013, page 2, book 3).

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Conflicts of interest

The authors declare to have no conflict of interest.

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None.

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