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Selective TNF- α targeting with infliximab attenuates impaired oxygen metabolism and contractile function induced by an acute exposure to air particulate matter

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Marchini T, D'Annunzio V, Paz ML, Cáceres L, Garcés M, Perez V, Tasat D, Vanasco V, Magnani N, Gonzalez Maglio D, Gelpi RJ, Alvarez S, Evelson P. Selective TNF- α targeting with infliximab attenuates impaired oxygen metabolism and contractile function induced by an acute exposure to air particulate matter. *Am J Physiol Heart Circ Physiol* 309: H1621–H1628, 2015. First published September 18, 2015; doi:10.1152/ajpheart.00359.2015.— Inflammation plays a central role in the onset and progression of cardiovascular diseases associated with the exposure to air pollution particulate matter (PM). The aim of this work was to analyze the cardioprotective effect of selective TNF- α targeting with a blocking anti-TNF- α antibody (infliximab) in an in vivo mice model of acute exposure to residual oil fly ash (ROFA). Female Swiss mice received an intraperitoneal injection of infliximab (10 mg/kg body wt) or saline solution, and were intranasally instilled with a ROFA suspension (1 mg/kg body wt). Control animals were instilled with saline solution and handled in parallel. After 3 h, heart O₂ consumption was assessed by high-resolution respirometry in left ventricle tissue cubes and isolated mitochondria, and ventricular contractile reserve and lusitropic reserve were evaluated according to the Langendorff technique. ROFA instillation induced a significant decrease in tissue O₂ consumption and active mitochondrial respiration by 32 and 31%, respectively, compared with the control group. While ventricular contractile state and isovolumic relaxation were not altered in ROFA-exposed mice, impaired contractile reserve and lusitropic reserve were observed in this group. Infliximab pretreatment significantly attenuated the decrease in heart O₂ consumption and prevented the decrease in ventricular contractile and lusitropic reserve in ROFA-exposed mice. Moreover, infliximab-pretreated ROFA-exposed mice showed conserved left ventricular developed pressure and cardiac O₂ consumption in response to a β -adrenergic stimulus with isoproterenol. These results provides direct evidence linking systemic inflammation and altered cardiac function following an acute exposure to PM and contribute to the understanding of PM-associated cardiovascular morbidity and mortality.

air pollution; residual oil fly ash; inflammation; infliximab; heart

NEW & NOTEWORTHY

The present study shows that impaired cardiac oxygen metabolism and contractile function induced by an acute exposure to air pollution particulate matter can be attenuated by blocking TNF- α -dependent systemic inflammation with infliximab, which emphasizes the importance of environmental factors and inflammation in cardiovascular disease.

NUMEROUS EPIDEMIOLOGICAL STUDIES have shown that decreased air quality levels are associated with increased morbidity and mortality rates due to respiratory and cardiovascular diseases (8). In its last report, the World Health Organization (WHO) estimated that 7 million people die per year as a result of the exposure to air pollution. In this scenario, cardiovascular diseases (ischemic heart disease and stroke) account for 69% of total death counts, compared with 31% of total mortality attributable to respiratory diseases (chronic obstructive pulmonary disease, acute lower respiratory disease, and lung cancer) (57).

Besides the complex nature of air pollution, and the coexistence of many compounds that may together contribute to the observed negative health impact, substantial epidemiological data point out that particulate matter (PM) is mainly responsible for the health outcomes (7). Nevertheless, the mechanisms by which PM exposure increases cardiovascular morbidity and mortality rates are poorly understood.

It is well established that human exposure to PM induces lung inflammation (1). Moreover, it has been reported that PM inhalation positively correlates with increased circulating pro-inflammatory mediators (10, 44, 52). As a consequence, many authors suggest that PM-associated cardiovascular diseases are a consequence of an immune response that is not limited to the lung but is able to progress to systemic inflammation, leading to altered cardiac function (7, 34, 50).

Airborne PM varies in size, chemical composition, and sources of origin. Anthropogenic emissions are the main contributors to air PM burden and mainly consist of motor vehicle emissions and fossil fuel combustion during power generation and industrial processes (38). The inorganic residue that remains after the incomplete oxidation of such carbonaceous materials contributes to PM in urban air and is termed residual oil fly ash (ROFA) (19). It has long been reported that an acute ROFA exposure leads to lung injury in humans (24) and mice

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(14, 20), as well as to increased circulating proinflammatory markers (34, 40) and diverse negative effects over the cardiovascular system (17, 34, 56). Nevertheless, direct evidence unraveling the mechanism by which PM-induced systemic inflammation is associated with altered cardiac function is scarce.

TNF- α is a pleiotropic cytokine, which plays a major role in the pathogenesis of numerous inflammatory diseases. It is suggested that TNF- α also mediates cardiac dysfunction during cardiovascular diseases (18). Interestingly, several studies have shown increased TNF- α levels in lung (16, 33), plasma (10), and cardiac tissue (29) following PM inhalation. In the present work, we hypothesize that TNF- α drives systemic inflammation after PM exposure and that this scenario negatively impacts over cardiac contractile function. Therefore, we aimed to analyze the cardioprotective effect of selective TNF- α targeting with a blocking anti-TNF- α antibody (infliximab) in an *in vivo* mice model of acute exposure to ROFA.

MATERIALS AND METHODS

Particulate matter. ROFA particles were collected from Boston Edison, Mystic Power Plant (Mystic, CT), burning low-sulfur residual oil (No. 6 fuel oil), and were kindly provided by Dr. J. Godleski (Harvard School of Public Health, Harvard University, Boston, MA). ROFA samples from this source have been extensively characterized in regard to their chemical composition and particle size. Vanadium, nickel, and iron are the predominant metals present as water-soluble sulfates, and the particle mean aerodynamic diameter is $2.06 \pm 1.57 \mu\text{m}$ (41). ROFA samples were freshly prepared by suspending particles in sterile saline solution at 0.5 mg/ml, followed by incubation in an ultrasonic water bath for 5 min before use.

Animal model of exposure to PM. Female Swiss mice weighing 20–25 g were randomized, anesthetized by an intraperitoneal injection of ketamine (10 mg/kg) and xylazine (0.1 mg/kg), and exposed to ROFA particles (1 mg/kg body wt) or saline solution (control group) in a single dose by intranasal instillation. Mice were immobilized in a 60° inclined supine position, while 50 μl of the ROFA suspension was delivered dropwise to the nares by the use of an automatic pipette. After 3 h, animals were euthanized in a CO₂ chamber and heart and blood samples were collected for analysis. Control mice were handled in parallel, instilled with 50 μl of sterile saline solution, and killed at the same time point. Because of the presence of fluid in the mouse nasal cavity, a respiratory reflex is triggered, which ensures that the maximum delivered volume reaches the lung (47). The 3-h endpoint was selected taking into account the main findings of previous studies from our group (34, 35), where a maximum decrease in cardiac O₂ consumption together with the highest plasma levels of proinflammatory cytokines (TNF- α and IL-6) was observed. The selected dose falls within the range of concentrations consistently used in several animal studies (20, 32, 40). Normalizing to the surface area of the respiratory epithelia (48), and considering air PM concentrations during atmospheric temperature inversions, peak hourly PM levels within certain megalopolis, and/or occupational exposures (from $\approx 100 \mu\text{g}/\text{m}^3$ and up to 200–500 $\mu\text{g}/\text{m}^3$) (6), the selected ROFA dose would represent a weekly human exposure to these highly polluted environments. Animal treatment was carried out following the 6344/96 regulation of the Argentinean National Drug, Food, and Medical Technology Administration (ANMAT) guidelines.

Infliximab pretreatment. Infliximab (10 mg/kg body wt; Remicade; Janssen Biotech, Horsham, PA) was injected intraperitoneally 30 min before the exposure to ROFA particles.

Cytokines quantification. Inflammatory cytokine levels were determined in plasma samples by ELISA. The OptEIA system (BD, Franklin Lakes, NJ) was used for TNF- α and IL-6 according to

manufacturer's instructions. Results were expressed as picograms per milliliters.

Cardiac O₂ consumption by left ventricle tissue cubes. Hearts were kept in Krebs buffer solution [118.5 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 1.5 mM CaCl₂, 24.8 mM NaHCO₃, and 10 mM glucose (pH 7.4)] at 4°C, and the left ventricle was isolated from the rest of the sample. After being washed and weighed, 1-mm³ tissue cubes were cut by the use of a scalpel. A Clark-type O₂ electrode (Hansatech Oxygraph, Hansatech Instruments, Norfolk, England) for high-resolution respirometry was used. Reaction buffer consisted of 118.5 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 24.8 mM NaHCO₃, and 5.5 mM glucose (pH 7.4). After an initial stabilization period, three to five tissue cubes were added to the reaction chamber and O₂ consumption rates were recorded at 30°C. Results were expressed as nanograms at oxygen per minute per grams of tissue (54).

Mitochondrial isolation. Heart mitochondrial purified fractions were obtained as described earlier (30) from left ventricle tissue homogenates and differential centrifugation in a Sorvall RC5C centrifuge (Sorvall, Buckinghamshire, England). Briefly, two mouse hearts were pooled and left ventricles were isolated, washed, and minced in ice-cold STE buffer [250 mM sucrose, 5 mM Tris-HCl, and 2 mM EGTA (pH 7.4)]. A brief digestion was performed in STE medium supplemented with 0.5% (wt/vol) fatty acid-free BSA, 5 mM MgCl₂, 1 mM ATP, and 2.5 UI/ml type XXIV bacterial proteinase. After 4 min at 4°C, samples were homogenized in 1:10 STE buffer with a Potter Elvehjem glass homogenizer and centrifuged at 8,000 *g* for 10 min. The obtained pellet was resuspended in STE buffer and centrifuged at 700 *g* for 10 min. The sediment was discarded and mitochondria were pelleted from the supernatant by two centrifugation steps at 8,000 *g* for 10 min each. Finally, the pellet was washed, rinsed, and resuspended in 500 μl of STE buffer. The whole procedure was carried out at 0–4°C. Protein concentration was measured by the Lowry assay (31) using BSA as a standard.

Mitochondrial respiration. The assessment of mitochondrial O₂ consumption rates in different metabolic states is the classical approach to evaluate mitochondrial function. A Clark-type O₂ electrode (Hansatech Oxygraph) for high-resolution respirometry was used. Freshly isolated mitochondria (0.15 mg protein/ml) were incubated at 25°C in respiration buffer [120 mM KCl, 5 mM KH₂PO₄, 1 mM EGTA, 3 mM HEPES, and 1 mg/ml fatty acid-free BSA (pH 7.2)] supplemented with 2 mM malate plus 5 mM glutamate. An initial resting respiration (state 4) was established under these conditions, which was then switched to active respiration (state 3) by the addition of 125 μM ADP. Results were expressed as nanograms at oxygen per minute per milligrams of protein. Respiratory control ratio (RCR) was calculated as state 3/state 4 respiration rates (55).

Isolated heart perfusion. Mice were anesthetized by an intraperitoneal injection of pentobarbital sodium (150 mg/kg body wt) and heparin sodium (500 UI/kg body wt). After sufficient depth of anesthesia was ensured, hearts were excised and the aorta was immediately cannulated with a 21-gauge cannula. Afterwards, hearts were perfused according to the Langendorff technique at constant flow (42) with Krebs medium [118.5 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 1.5 mM CaCl₂, 24.8 mM NaHCO₃, 10 mM glucose (pH 7.4)] equilibrated with 95% O₂-5% CO₂ at 37°C. A small latex fluid-filled balloon was connected via a thin plastic catheter (P50) to a Deltram II pressure transducer (Utah Medical System) and inserted into the left ventricle via the left atrium. The catheter with the transducer was positioned in such a way that it secured the position of the balloon in the left ventricle. Two electrodes were sutured and connected to a pacemaker to induce a constant heart rate of 470 ± 30 beats/min. To record coronary perfusion pressure (CPP), a second pressure transducer was connected to the perfusion line. Hearts were perfused at constant flow to obtain a CPP of 73 ± 3 mmHg during the initial stabilization period and then maintained constant throughout the experiment. Left ventricular developed pressure (LVDP) was

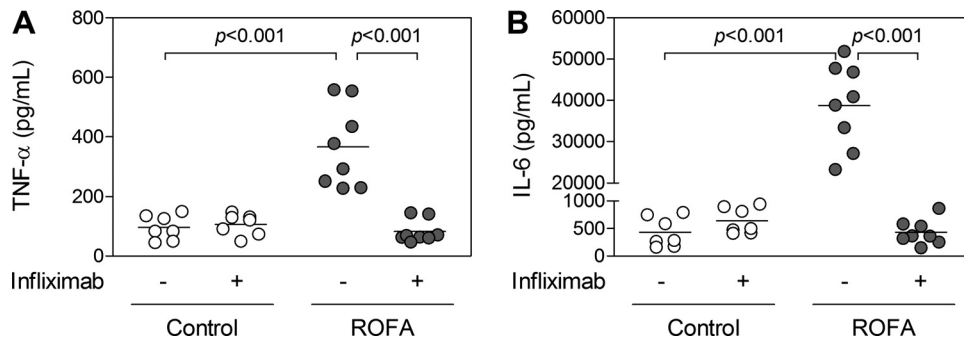


Fig. 1. Infiximab pretreatment attenuates systemic inflammation in residual oil fly ash (ROFA)-exposed mice. TNF- α (A) and IL-6 (B) plasma levels were evaluated by ELISA in plasma samples from mice exposed to saline solution (white circles) or a ROFA suspension (grey circles), and with or without infiximab pretreatment.

calculated as the difference between peak systolic pressure and left ventricular end-diastolic pressure. Isovolumic relaxation (lusitropism) was analyzed as the time required for the left ventricular pressure to fall up to 50% from the peak of LVDP (t_{50}) (49). Ventricular contractile reserve and lusitropic reserve were evaluated as LVDP and t_{50} , before and after a β -adrenergic stimulus through the addition of isoproterenol (ISO; 1 μ M) to the perfusion line (34). The mentioned procedure was also performed in perfused hearts with a low-calcium Krebs buffer [118.5 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 , 1.25 mM CaCl_2 , 24.8 mM NaHCO_3 , and 10 mM glucose (pH 7.4)].

Statistics. Data are presented as means \pm SE. Unpaired Student's t -test was used to analyze differences between two groups. ANOVA followed by the post hoc Student-Newman-Keuls test was performed to analyze differences between more than two groups. Statistical significance was considered at $P < 0.05$.

RESULTS

Cytokine quantification. As shown in Fig. 1, TNF- α and IL-6 plasma levels were significantly increased in ROFA-exposed mice compared with the control group ($P < 0.001$). As expected, infiximab pretreatment prevented this effect. Both TNF- α and IL-6 levels in infiximab-pretreated ROFA-exposed mice were significantly decreased compared with ROFA-exposed mice that did not receive infiximab ($P < 0.001$). No significant differences were observed among the control groups with or without infiximab. This result indicates that blocking TNF- α biological activity with infiximab prevents systemic inflammation in ROFA-exposed mice.

Cardiac O_2 consumption. Figure 2A shows a representative measurement of O_2 consumption rates by left ventricle tissue cubes from the different experimental groups. As shown in Fig. 2B, cardiac O_2 consumption was significantly decreased by

32% in ROFA-exposed mice compared with the control group ($P < 0.01$). Infiximab pretreatment reduced this effect, as cardiac O_2 consumption was significantly increased in infiximab-pretreated ROFA-exposed mice compared with ROFA-exposed mice that did not receive infiximab ($P < 0.05$). No significant differences were observed among the control groups with or without infiximab. Even though no statistical significance was observed, a 14% decrease remained when comparing O_2 consumption rates from infiximab-pretreated ROFA-exposed mice with the control group. These results indicate that TNF- α -dependent systemic inflammation accounts, at least in part, for the decrease in cardiac O_2 consumption induced by an acute ROFA exposure.

Mitochondrial O_2 consumption. Under physiological conditions, 85–90% of tissue O_2 uptake is consumed by mitochondria in the oxidative phosphorylation process (4). Moreover, it has been reported that TNF- α (intraperitoneal) alters mitochondrial function in rat heart (36). Therefore, mitochondrial respiration was evaluated to deepen the study of the observed changes in heart O_2 metabolism in ROFA-exposed mice, as well as the contribution of TNF- α -dependent systemic inflammation in this scenario. Figure 3A shows a representative measurement of mitochondrial O_2 consumption under resting (state 4) and active (state 3) respiration in isolated mitochondria from the left ventricle of hearts from the different experimental groups. As shown in Fig. 3B, state 3 respiration rate was significantly decreased by 31% in ROFA-exposed mice compared with the control group ($P < 0.05$). Consequently, RCR was also decreased in this group due to decreased state 3 and unchanged state 4 respiration rates (Fig. 3C). Infiximab pretreatment reduced this effect, as state 3 respiration rate and RCR were significantly increased in infiximab-pretreated

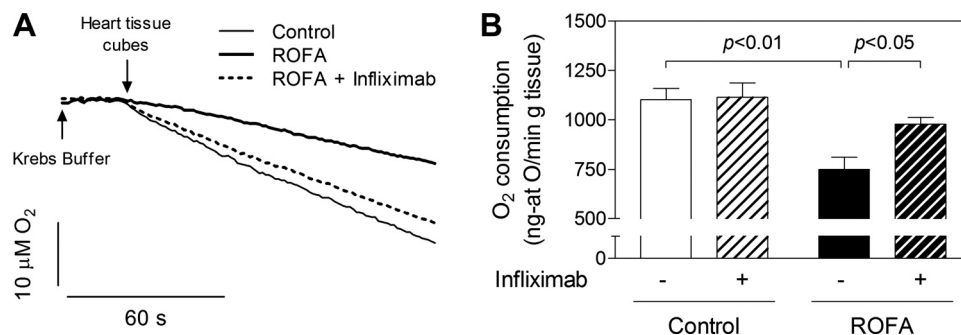


Fig. 2. Infiximab pretreatment attenuates decreased cardiac O_2 metabolism in ROFA-exposed mice. A: representative traces obtained during the assessment of O_2 consumption by high-resolution respirometry in left ventricle tissue cubes from mice exposed to saline solution (thin line) or a ROFA suspension (thick line), and pretreated with infiximab (hatched line). B: cardiac O_2 consumption rates in mice exposed to saline solution (white bars) or a ROFA suspension (black bars), and pretreated with infiximab (hatched bars). Data are presented as means \pm SE of at least 6 independent experiments.

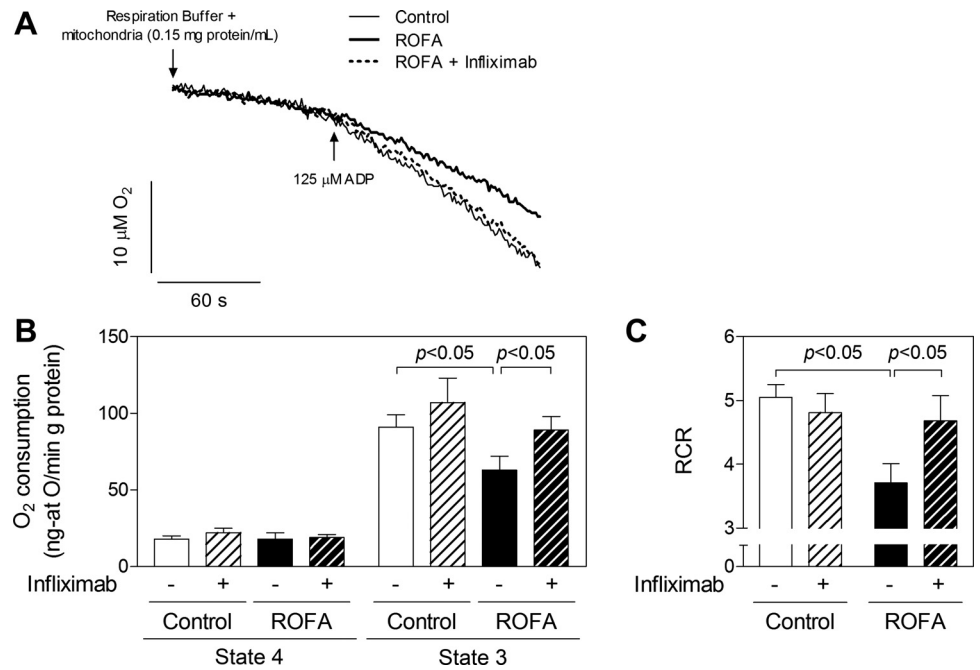


Fig. 3. Infiximab pretreatment attenuates impaired mitochondrial function in ROFA-exposed mice. A: representative traces obtained during the assessment of mitochondrial respiration in resting and active metabolic states by high-resolution respirometry. Mitochondria were isolated from the left ventricle of mice exposed to saline solution (thin line) or a ROFA suspension (thick line), and pretreated with infiximab (hatched line). B: resting (state 4) and active (state 3) O₂ consumption rates of isolated mitochondria from mice exposed to saline solution (white bars) or a ROFA suspension (black bars), and pretreated with infiximab (hatched bars). C: respiratory control ratios (RCR), calculated as state 3/state 4 O₂ consumption rates from the same experimental group. Data are presented as means ± SE of at least 6 independent experiments.

ROFA-exposed mice compared with ROFA-exposed mice that did not receive infiximab ($P < 0.05$). No significant differences were observed among control groups with or without infiximab or in state 4 respiration rates between the different groups. These results suggest that increased circulating TNF- α levels in ROFA-exposed mice lead to impaired mitochondrial function, which might, in turn, account for decreased cardiac tissue O₂ consumption in this group.

Ventricular contractile reserve. As shown in Table 1, LVDP was not altered among the different experimental groups in basal conditions. However, after a β -adrenergic stimulus with ISO, the increase in LVDP (Δ LVDP) was significantly attenuated in ROFA-exposed mice compared with the control group. While a 52% increase was observed in LDVP after ISO perfusion in the control group, Δ LVDP was only 18% in ROFA-exposed mice ($P < 0.01$). Interestingly, Δ LVDP was 35% in infiximab-pretreated ROFA-exposed mice, indicating that infiximab ameliorates the decrease in ventricular contrac-

tile reserve following an acute ROFA exposure. No significant differences were observed in Δ LVDP between the control groups with or without infiximab. Even though no statistical significance was observed compared with the control group, the decrease in Δ LVDP was not completely abolished by infiximab pretreatment in ROFA-exposed mice.

Ventricular lusitropic reserve. As shown in Table 2, t_{50} was also not altered among the different experimental groups in basal conditions. However, after ISO perfusion, the decrease in t_{50} (Δt_{50}) was significantly attenuated in ROFA-exposed mice compared with the control group. While a 31% decrease was observed in t_{50} after ISO perfusion in the control group, Δt_{50} was only -3% in ROFA-exposed mice ($P < 0.05$). Interestingly, Δt_{50} was -21% in infiximab-pretreated ROFA-exposed mice, indicating that infiximab also attenuates the decrease in ventricular lusitropic reserve following an acute ROFA exposure. No significant differences were observed in Δt_{50} between the control groups with or without infiximab. Even though no

Table 1. Evaluation of LVDP in basal conditions and after a β -adrenergic stimulus with ISO according to the Langendorff technique at constant flow

	LVDP, mmHg		Δ LVDP, %
	Basal	ISO	
Control			
-Infiximab	82 ± 5	114 ± 8*	52 ± 9
+Infiximab	84 ± 6	121 ± 9*	53 ± 11
ROFA			
-Infiximab	86 ± 9	102 ± 11	18 ± 3†
+Infiximab	95 ± 5	129 ± 7*	35 ± 6‡

Data are presented as means ± SE of at least 6 independent experiments. Ventricular contractile reserve (Δ LVDP) was calculated as the percentage increase in left ventricular developed pressure (LVDP) after isoproterenol (ISO) perfusion. * $P < 0.001$, compared with the corresponding LVDP in basal conditions for each group. † $P < 0.01$, compared with Δ LVDP in the control group without Infiximab pretreatment. ‡ $P < 0.01$, compared with Δ LVDP in ROFA-exposed mice without Infiximab pretreatment.

Table 2. Evaluation of t_{50} in basal conditions and after a β -adrenergic stimulus with ISO according to the Langendorff technique at constant flow

Infiximab	t_{50} , ms		Δt_{50} , %
	Basal	ISO	
Control			
-Infiximab	14 ± 1	10 ± 1*	-31 ± 5
+Infiximab	16 ± 1	11 ± 1*	-33 ± 6
ROFA			
-Infiximab	15 ± 3	14 ± 3	-3 ± 9†
+Infiximab	16 ± 5	11 ± 3	-21 ± 7‡

Data are presented as means ± SE of at least 6 independent experiments. Ventricular lusitropic reserve (Δt_{50}) was calculated as the percentage decrease in t_{50} after ISO perfusion. * $P < 0.01$, compared with the corresponding t_{50} in basal conditions for each group. † $P < 0.05$, compared with Δt_{50} in the control group without Infiximab pretreatment. ‡ $P < 0.05$, compared with Δt_{50} in ROFA-exposed mice without Infiximab pretreatment.

statistical significance was observed compared with the control group, the decrease in Δt_{50} was not completely abolished by infliximab pretreatment in ROFA-exposed mice.

Cardiac O_2 consumption after ISO perfusion. Following isolated heart perfusion experiments, hearts were removed from the Langendorff apparatus and O_2 consumption rates were assessed in left ventricle tissue cubes (Fig. 4). ISO perfusion induced a 44% increase in cardiac O_2 consumption when compared with basal conditions in the control group ($P < 0.001$). When mice were instilled with the ROFA suspension, ISO perfusion increased O_2 consumption by 32% ($P < 0.05$). Infliximab pretreatment partially restored O_2 consumption rates in response to ISO perfusion, given that ISO perfusion increased cardiac O_2 consumption by 37% in ROFA-exposed mice ($P < 0.01$). In terms of absolute values, cardiac O_2 consumption rate after ISO perfusion was decreased by 41% in ROFA-exposed mice that did not receive infliximab when compared with the control group ($P < 0.001$). Although it was not completely abolished, infliximab pretreatment reduced this effect, as cardiac O_2 consumption after ISO perfusion was only decreased by 17% in infliximab-pretreated ROFA-exposed mice compared with the control group ($P < 0.05$). No significant differences were observed in O_2 consumption rates between the control groups with or without infliximab, both in basal conditions or after ISO perfusion. Taken together, these results suggest that infliximab pretreatment improved not only cardiac O_2 metabolism in basal conditions but also after a β -adrenergic stimulus in ROFA-exposed mice.

Isolated heart perfusion with a low-calcium Krebs buffer. It has been shown that TNF- α alters cardiomyocyte calcium homeostasis, leading to myocardial dysfunction during inflammation (22, 51). To evaluate the role of calcium in the observed changes in cardiac function of ROFA-exposed mice, left ventricular contractile reserve and lusitropic reserve were evaluated in hearts perfused with a low-calcium Krebs buffer. As shown in Tables 3 and 4, cardiac contractile function and isovolumic relaxation in ROFA-exposed mice resembled to that of hearts from the control group. Both showed signifi-

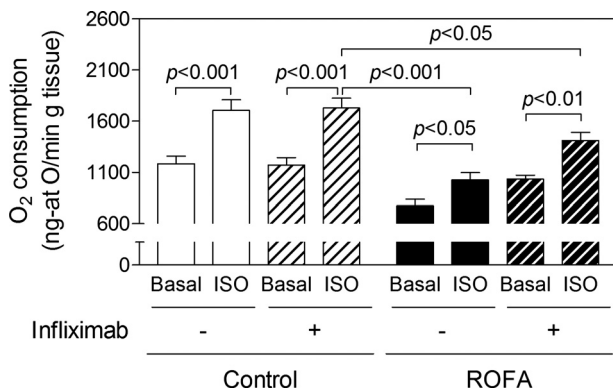


Fig. 4. Infliximab pretreatment attenuates decreased cardiac O_2 metabolism in ROFA-exposed mice, both in basal conditions and after ISO perfusion. Heart samples were obtained from mice exposed to saline solution (white bars) or a ROFA suspension (black bars), and pretreated with infliximab (hatched bars). Measurements were performed after the evaluation of cardiac function by the Langendorff technique in basal conditions and after a β -adrenergic stimulus induced by ISO perfusion. Data are presented as means \pm SE of at least 6 independent experiments.

Table 3. Evaluation of LVDP in basal conditions and after a β -adrenergic stimulus with ISO according to the Langendorff technique at constant flow with a low calcium-Krebs buffer

	LVDP, mmHg		Δ LVDP, %
	Basal	ISO	
Control	87 \pm 3	159 \pm 8*	65 \pm 13
ROFA	88 \pm 3	151 \pm 9*	80 \pm 18

Data are presented as means \pm SE of at least 6 independent experiments. Ventricular contractile reserve (Δ LVDP) was calculated as the percentage increase in LVDP after ISO perfusion. * $P < 0.001$, compared with the corresponding LVDP in basal conditions for each group.

cantly increased LVDP and decreased t_{50} following ISO perfusion compared with basal conditions, as well as conserved Δ LVDP and Δt_{50} . This recovery of cardiac contractile and lusitropic reserve when hearts were perfused with a low-calcium Krebs buffer suggests that altered calcium homeostasis might be involved in TNF- α deleterious effect over ventricular function in ROFA-exposed mice.

DISCUSSION

Numerous epidemiological studies have shown that the exposure to environmental PM positively correlates with increased cardiovascular morbidity and mortality rates (7). We and others have previously reported that PM exposure induces lung and systemic inflammation (19, 35, 40), as well as adverse cardiovascular effects (17, 34). Therefore, a first local and then systemic immune response has been postulated as the underlying mechanism of PM-associated cardiovascular diseases. In this study, we provide direct evidence that cardiac dysfunction following PM inhalation is mediated, at least in part, by TNF- α -driven systemic inflammation.

Diverse PM surrogates have been assayed in different animal models to study the biological effects of PM exposure. It has been suggested that the presence of soluble transition metals as PM constituents enhance the inflammatory response triggered by PM because of increased production of reactive oxygen species via Fenton-like chemical reactions (12). In this context, ROFA has been particularly useful given that it is especially rich in soluble transition metals (namely iron, nickel, and vanadium) and because of its low concentration of organic compounds (15). Interestingly, under the same experimental conditions, it has been shown that ROFA induces a greater TNF- α release from RAW 264.7 cells than carbon black

Table 4. Evaluation of t_{50} in basal conditions and after a β -adrenergic stimulus with ISO according to the Langendorff technique at constant flow with a low calcium-Krebs buffer

	t_{50} , ms		Δt_{50} , %
	Basal	ISO	
Control	20 \pm 2	14 \pm 2*	-22 \pm 3
ROFA	17 \pm 1	14 \pm 1*	-21 \pm 2

Data are presented as means \pm SE of at least 6 independent experiments. Ventricular lusitropic reserve (Δt_{50}) was calculated as the percentage decrease in t_{50} after ISO perfusion. * $P < 0.01$, compared with the corresponding t_{50} in basal conditions for each group.

particles (43). In vivo studies have also reported a higher increase in cardiovascular toxicity following ROFA inhalation compared with carbon black exposure (23, 56). Such differential biological effects are frequently attributed to the presence of different transition metals in ROFA (12, 14, 15). In addition, ROFA particles often present an aerodynamic diameter $\leq 2.5 \mu\text{m}$ ($\text{PM}_{2.5}$), a size that has been shown to be more closely associated with PM adverse health effects than coarse particles ($\text{PM}_{10-2.5}$) (7).

Infliximab is a chimeric (mouse/human) monoclonal antibody that blocks TNF- α biological activity by binding with high affinity and specificity to both the soluble and membrane-bound forms of TNF- α (26). Infliximab is currently employed in the clinic for the treatment of TNF- α -mediated inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease, among others (46). In the present work, we used infliximab to test the hypothesis that TNF- α orchestrates systemic inflammation leading to impaired cardiac function following PM exposure.

As expected, infliximab pretreatment attenuated the increase in plasma TNF- α and IL-6 triggered by ROFA instillation (Fig. 1). Using the same animal model, we have previously reported that cardiac O_2 consumption is decreased in ROFA-exposed mice (34), together with increased TNF- α and IL-6 levels in lung and plasma (35). These results suggested that depressed cardiac metabolism might be associated with systemic inflammation following ROFA instillation. In the present work, we show that infliximab pretreatment attenuates the decrease in cardiac tissue O_2 consumption in ROFA-exposed mice (Fig. 2). Furthermore, ROFA exposure also induced a decrease in mitochondrial active respiration rate and RCR (Fig. 3), a well-established indicator of mitochondrial function (5), which might explain the decrease in whole tissue cardiac O_2 consumption in this group. Interestingly, altered mitochondrial function in ROFA-exposed mice was attenuated by infliximab pretreatment. Taken together, these findings suggest that TNF- α -mediated systemic inflammation accounts, at least in part, for the observed decrease in heart O_2 consumption in ROFA-exposed mice due to mitochondrial function impairment. In support of this concept, it has been reported that intraperitoneal TNF- α induces mitochondrial dysfunction and decreased O_2 consumption in rat left ventricle, impairing cardiac contractile function (37). In this context, increased nitric oxide (NO) production in the heart as a consequence of PM-induced systemic inflammation might play a role. NO is a well-known inhibitor of tissue O_2 consumption (3), and it is synthesized in large quantities by inducible nitric oxide synthase (iNOS) during inflammation (55). Moreover, it has been shown that PM exposure in rodents leads to increased iNOS expression and NO production in heart (29), and to increased NO production by circulating polymorphonuclear leucocytes (35). NO modulates mitochondrial respiration by a competitive inhibition of cytochrome *c* oxidase with O_2 at the inner mitochondrial membrane (2). In line with this concept, we have previously described that an acute ROFA exposure leads to decreased O_2 metabolism in heart (34) and showed in the present work that infliximab pretreatment attenuated this effect in ROFA-exposed mice (Figs. 2 and 3). The inhibition of the inflammatory response due to TNF- α blockage might reduce NO levels and, in turn, restore cardiac tissue and mitochondrial O_2 consumption rates in this group.

Taking into account that cardiac O_2 metabolism is not completely restored by infliximab pretreatment in ROFA-exposed mice, alternative biological mechanisms might contribute to this scenario. It has been shown that nanoscale particles and/or soluble PM constituents are able to break through the respiratory epithelia, translocate into systemic circulation, and reach the heart within minutes to hours after PM inhalation (39). Consequently, a direct effect of ROFA over heart tissue cannot be ruled out. In support of this concept, it has been reported that neonatal rat cardiomyocytes incubated with particle-free leachates of ROFA exhibited signs of cardiotoxicity (27). Moreover, it has been recently shown that PM is able to alter contractile function and calcium handling, not only by indirect mechanisms involving proinflammatory mediators, but also in a direct fashion as well (21).

Systemic immune response following PM inhalation is suggested to occur as a consequence of local inflammation due to the presence of PM within the lung (7, 53). Accordingly, increased proinflammatory cytokine levels have been reported in lung and bronchoalveolar lavage fluid from ROFA-exposed mice (20, 35, 45) and confirmed in vitro (11, 53). The release of these immune mediators into the blood stream might drive the immune response leading to systemic inflammation, which has been suggested to be associated with the observed adverse cardiovascular effects after PM inhalation (25, 28, 35). In line with this concept, we showed in the present work that cardiac dysfunction in ROFA-exposed mice is attenuated by blocking TNF- α -dependent systemic inflammation with infliximab. As shown in Tables 1 and 2, infliximab pretreatment reduced both the decrease in ventricular contractile and lusitropic reserve in ROFA-exposed mice, as well as the decrease in cardiac O_2 metabolism in basal conditions and after a β -adrenergic stimulus with ISO (Fig. 4). In addition, we have previously reported that cardiac contractility positively correlates with heart O_2 consumption in a normal myocardium, which is lost in hearts from ROFA-exposed mice (35). These results indicate that the myocardium fails to properly sustain contractile work when work output is increased in mice exposed to PM. Interestingly, infliximab pretreatment recovered the positive correlation between cardiac contractile state and O_2 consumption (Fig. 5), suggesting that blocking TNF- α -dependent systemic inflammation with infliximab prevents the heart from failing to increase the contractile state when energy requirements rise in

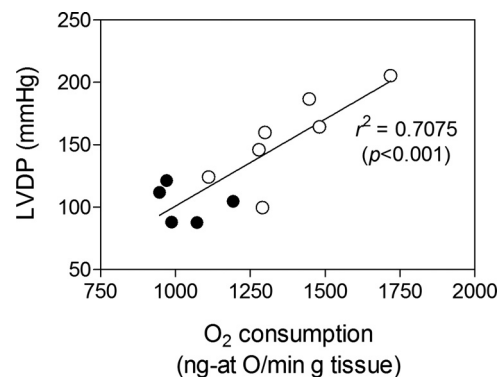


Fig. 5. Linear correlation between left ventricle O_2 consumption and left ventricular developed pressure (LVDP) in infliximab-pretreated ROFA-exposed mice. Measurements were performed in basal conditions (●) and after β -adrenergic stimulus by ISO perfusion (○).

ROFA-exposed mice. Together with impaired mitochondrial function, altered myocardial calcium homeostasis in ROFA-exposed mice might contribute to the present findings, as cardiac systolic and diastolic function also recovers when hearts were perfused with a low-calcium Krebs buffer (Tables 3 and 4).

Although several reports indicate that TNF- α is detrimental for proper cardiac function (9), clinical trials in heart failure patients using TNF- α inhibitors, such as infliximab, have been disappointing (13). In this work, we did not deliver infliximab as a potential therapeutic strategy for PM-associated cardiovascular diseases but identified a putative mechanism linking systemic inflammation and impaired cardiac function following PM exposure. To the best of our knowledge, this is the first time in which such direct evidence is reported. Taken together, the present results indicate that blocking TNF- α biological activity attenuates myocardial dysfunction induced by acute PM exposure. This finding emphasizes the importance of environmental factors and inflammation in cardiovascular disease, and contributes to the understanding of increased cardiovascular morbidity and mortality rates in association with the exposure to air pollution PM.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: T.M., V.D., M.L.P., L.C., and M.G. performed experiments; T.M., V.D., and M.L.P. interpreted results of experiments; T.M. prepared figures; T.M. drafted manuscript; V.P., V.V., and N.M. analyzed data; D.T., D.G.M., R.J.G., S.A., and P.E. approved final version of manuscript; D.G.M., R.J.G., and P.E. conception and design of research; D.G.M., R.J.G., S.A., and P.E. edited and revised manuscript.

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