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The anti-inflammatory and anti-oxidative actions of eugenol improve lipopolysaccharide-induced lung injury

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ABSTRACT

Acute lung injury (ALI) remains a major cause of mortality. In lipopolysaccharide (LPS)-stimulated macrophages, eugenol reduces cyclooxygenase-2 expression, NF-κB activation, and inflammatory mediators. We examined the anti-inflammatory and anti-oxidative action of eugenol in an *in vivo* model of LPS-induced lung injury. Lung mechanics and histology were analyzed in mice 24 h after LPS exposure, with and without eugenol treatment at different doses. Additional animals, submited to the same protocol, were treated with eugenol at 150 mg/kg to determine its effect on inflammatory cytokines (ELISA) and oxidative markers. LPS-induced lung functional and histological changes were significantly improved by eugenol, in a dose-dependent way. Furthermore, eugenol (150 mg/kg) was able to inhibit the release of inflammatory cytokines (TNF-α, IL-1β and IL-6), NADPH oxidase activity, as well as antioxidant enzymes activity (superoxide dismutase, catalase and glutathione peroxidase). Finally, eugenol reduced LPS-induced protein oxidation. In conclusion, eugenol improved *in vivo* LPS-induced ALI through both anti-inflammatory and anti-oxidative effects, avoiding damage to lung structure.

1. Introduction

Acute lung injury (ALI), characterized by severe inflammation of lung parenchyma, remains a major cause of mortality in intensive care units (Orban et al., 2017). Although there is a better understanding of its physiopathology, no effective treatment is presently available. Lipopolysaccharide (LPS)-induced ALI is a well-established model that reproduces morphological and functional changes observed in ALI. LPS binds to toll-like receptor 4 (TLR4), triggering a cascade of inflammatory response that involves tumor necrosis factor-α (TNF-α) release and nuclear factor-κB (NF-κB) activation. Furthermore, reactive oxygen species (ROS) have also been implicated in TLR4 activation and signaling, regulating the inflammatory response as well as end-organ injury (Shi et al., 2010; Kong et al., 2010; Valença et al., 2008). NADPH oxidases (NOX) are the main source of ROS after LPS exposure (Bernard et al., 2014). NOX family is composed of seven members, NOX1-NOX5 and DUOX1/2, which are differentially expressed among tissues (Bedard and Krause, 2007). Several NOX enzymes are present in the airways and lung cells, where they are involved in a wide range of physiological processes. However, the overproduction of ROS by them is responsible for the tissue injury associated with chronic inflammatory diseases, leading to exaggerated or deregulated activation of the anti-oxidant defense system (Lambeth, 2004). Several studies suggest that redox events may modulate NF-κB activity (Janssen-Heininger et al., 2000; Ngkelo et al., 2012). In fact, cellular redox state is critical for innate immune regulation and cellular response to external stressors (Kolls, 2006). Therefore, ROS and cellular antioxidants may act as modifiers in ALI pathogenesis (Kolls, 2006; Kong et al., 2010).

Previous studies demonstrate that phenolic compounds, such as eugenol (4-allyl-2-methoxyphenol) - a component of clove oil commonly used in dentistry -, show anti-inflammatory and antioxidative activities (Murakami et al., 2003, 2005). *In vitro* data suggest that these compounds interfere with TNF signaling leading to NF-κB activation. Indeed, phenolic antioxidants inhibit nuclear factor-κB (NF-κB)

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activation induced by TNF-α in LPS-stimulated macrophages in culture (Ma and Kinneer, 2002; Murakami et al., 2003, 2005). In 2015, a preventive anti-oxidative effect of eugenol was also reported in an ALI model, however, neither the mechanisms involved nor the functional impact of eugenol were investigated (Huang et al., 2015). Based on these previous observations, our group examined the anti-inflammatory effects of eugenol in an *in vivo* model of LPS-induced lung injury. Our previous results showed that eugenol improves LPS-induced lung functional changes, as well as lung inflammation (Magalhães et al., 2010). The present work further investigates eugenol anti-oxidative effect and its functional relevance as treatment in LPS-induced ALI *in vivo*.

2. Materials and methods

2.1. Animal preparation

All animals received humane care and the experiments complied with the following guidelines: ARRIVE, the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978), and National Council for Controlling Animal Experimentation, Ministry of Science, Technology and Innovation (CONCEA/MCTI), Brazil. This study was approved by the Ethics Committee on the Use of Animals, Health Sciences Center, Federal University of Rio de Janeiro (Protocol IBCCF 050). The experimental study was carried on in a research laboratory.

A total of 145 mice was used in this study and below the number pertaining to each protocol is detailed. Male BALB/c mice (20–25 g) randomly received an intratracheal (*i.t.*) injection (through cervical incision) of 0.05 mL of sterile saline solution (0.9% NaCl) or 10 μg of lipopolysaccharide (*Escherichia coli* serotype O55:B5 – Sigma Chemical Co., St. Louis, MO, USA) in 0.05 mL of saline (groups C and L, respectively). This dose of LPS yields a 1.5-fold increase in lung static elastance (Est) compared with control animals (Menezes et al., 2005). Initially, we determined a dose-response curve for eugenol treatment. To that end, mice were treated with either 0.2 mL saline + tween 80 (1% vv) [C (n = 9) and L (n = 9) groups] or increasing doses of eugenol (15, 50, 100, 150, 500, 1000, and 1500 mg/kg) + tween 80 in a total volume of 0.2 mL (1% vv) by gavage (LE1-LE7 groups, n = 8–9/each) 6 h after saline or LPS injection. Lung mechanics and histology were analyzed 24 h after saline or LPS administration.

The lower effective eugenol dose – that reduced LPS-induced elastance changes back to control values - was used for the determination of inflammatory and oxidative stress markers, as follows. Additional mice underwent the aforementioned protocol of saline/LPS injection. Six hours later mice received either 0.2 mL saline + tween 80 (1% vv) [C and L groups (n = 10/each)] or eugenol (150 mg/kg) + tween 80 in 0.2 mL (1% vv) by gavage [E and LE groups (n = 10/each)]. Twenty-four hours after saline/LPS injection, left and right lungs were removed and their homogenates used for inflammatory cytokine or oxidative stress analysis, respectively.

2.2. Pulmonary mechanics

Lung resistive (ΔP1) and viscoelastic/inhomogeneous (ΔP2) pressures, as well as lung static elastance (E_{st}) and its viscoelastic component (ΔE) were computed by the end-inflation occlusion method, as previously described (Magalhães et al., 2010). ΔP1 selectively reflects airway resistance, and ΔP2 reflects stress relaxation, or viscoelastic properties of the lung (Bates et al., 1985). Lung mechanics was measured 10 times in each animal.

Briefly, mice were sedated (diazepam, 1 mg *i.p.*), anesthetized (pentobarbital sodium, 20 mg/kg body wt *i.p.*), paralyzed (pancuronium bromide, 0.1 mg/kg body wt *i.v.*), and mechanically ventilated (Samay VR15, Universidad de la Republica, Montevideo, Uruguay) with a frequency of 100 breaths/min, tidal volume of 0.2 mL, flow of 1 mL/s, and positive end-expiratory pressure of 2 cmH_2O. The anterior chest wall was surgically removed.

A pneumotachograph was connected to the tracheal cannula for airflow (V') measurement. Lung volume was obtained by digital integration of V' signal. The pressure gradient across the pneumotachograph was determined by means of a differential pressure transducer (Validyne MP-45-2, Validyne Engineering, Northridge, CA, USA). Equipment resistive pressure (= equipment resistance multiplied by V') was subtracted from pulmonary resistive pressure so that the present results represent intrinsic values. Transpulmonary pressure was measured with a Validyne MP-45 differential pressure transducer (Validyne Engineering, Northridge, CA, USA) (Faffe et al., 2000).

2.3. Histological study

After determination of pulmonary mechanics, heparin (1000 IU) was intravenously injected. Animals were euthanatized by exsanguination (sectioning of inferior vena cava and abdominal aorta). The trachea was clamped at end-expiration, lungs were isolated *en bloc* and fixed with formaldehyde 10% in Millonig's phosphate buffer (100 mL HCHO, 900 mL H_2O, 18.6 g NaH_2PO_4, 4.2 g NaOH). After fixation, lungs were routinely processed for histology (Magalhães et al., 2010). The tissue was embedded in paraffin, and, then, 4 μm-thick slices were cut and stained with hematoxylin and eosin (HE), as well as picrosirius for collagen fiber detection.

Fraction areas of normal and collapsed alveoli were determined at 200x magnification, as previously described (Magalhães et al., 2010), by the point-counting technique (Gundersen et al., 1988). Ten random non-overlapping microscopic fields/sample were analyzed on a conventional light microscope (Axioplan, Zeiss, Oberkochen, Germany). The total number of mononuclear (MN) and polymorphonuclear (PMN) cells per pulmonary tissue area was evaluated at 1,000x magnification. The interstitial area of lung parenchyma occupied by collagen fibers was quantified in picrosirius-stained sections across 10 high-definition images (2024 × 1536 pixels) from random non-coincident microscopic fields (400x magnification) using Image Pro Plus 4.5.1 (Media Cybernetics, Silver Spring, MD, USA). Results were expressed as percentage of collagen fiber area/pulmonary tissue area.

2.4. Cytokines

Lungs from other nine mice in each group (C, E, L, and LE) were prepared for cytokine determination in lung homogenates. For such purpose, left lungs were removed, homogeneized using a tissue mixer (Tissuemiser, Fisher Scientific, Hampton, NH, USA) in potassium phosphate buffer (100 mM plus EDTA 5 mM, in 1000 mL), and centrifuged at 7000 x g for 10 min at 4 °C (Centrifuge 541 R, Germany). Supernatant aliquots were kept at −80 °C. Protein concentration was determined by the Bradford method (Bradford, 1976). TNF-α, IL-1β, and IL-6 levels were quantified by ELISA (R&D Systems Inc., Minneapolis, MN, USA) in accordance with the manufacturer's instructions. Results were expressed as cytokine (pg) per mg of protein.

2.5. NADPH oxidase activity

Lungs from an extra group of six mice submitted to the same protocol described for cytokine determination were used for NOX activity quantification. Thus, NOX activity was determined by the Amplex red/horseradish peroxidase assay, which detects the accumulation of a fluorescent oxidized product, as previously described (Cardoso et al., 2002). Briefly, the particulate fraction of lung homogenates was obtained by centrifugation at 3000 × *g* for 15 min at 4 °C and resuspended in 0.5 mL 50 mM sodium phosphate buffer, pH 7.2, containing 0.25 M sucrose, 2 mM $MgCl_2$, 5 mg/mL aprotinin, and 34.8 mg/mL phenylmethanesulfonyl fluoride (PMSF) (buffer 1). The pellet was washed twice with buffer 1 and centrifuged at 3000 × *g* for 15 min at 4 °C. The

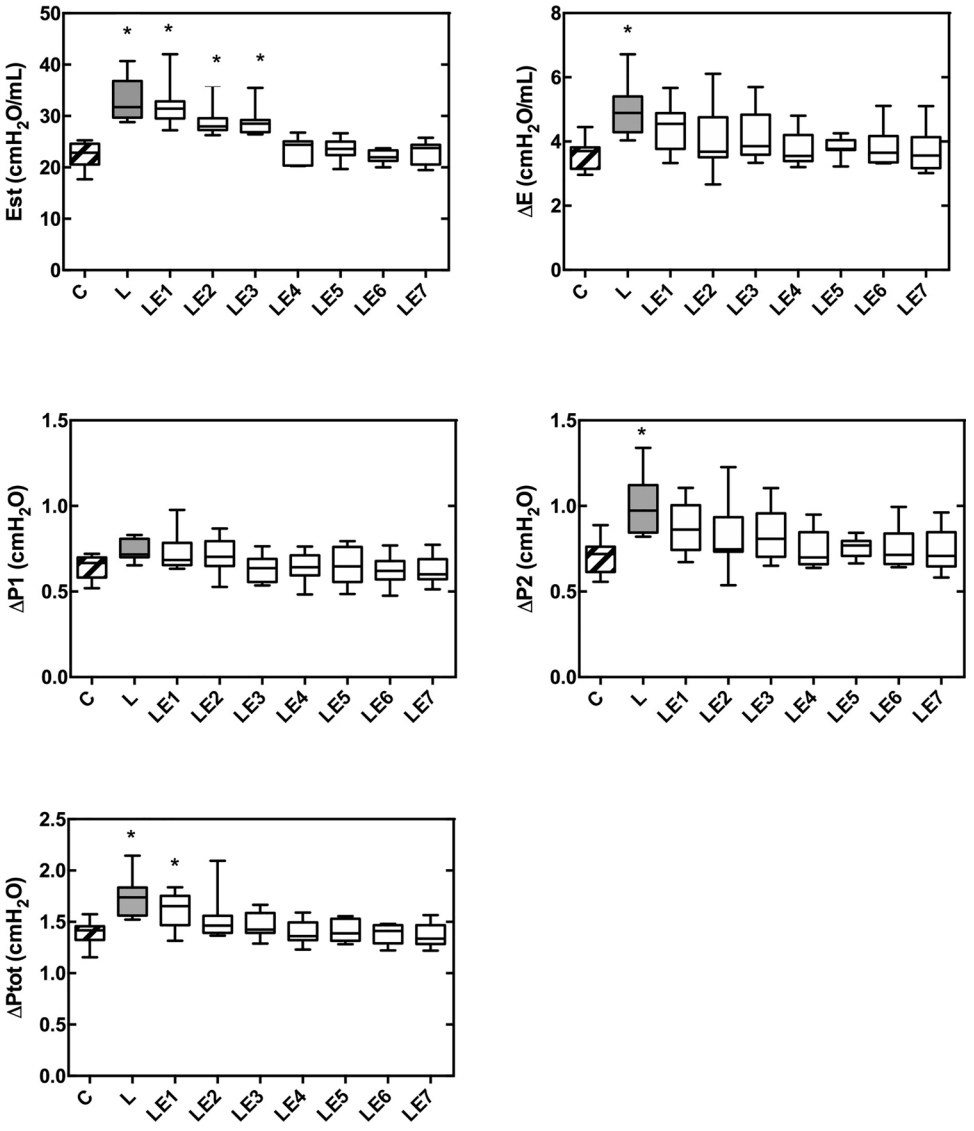

Fig. 1. Eugenol reduced LPS-induced changes in lung mechanics in a dose-dependent manner. Lung mechanics in mice 24 h after saline (C) or lipopolysaccharide (10 μg, L) intratracheal instillation, with and without treatment with increasing doses of eugenol (15, 50, 100, 150, 500, 1000, and 1500 mg/kg; groups LE1-7, respectively) by gavage 6 h after instilation. *Upper panels*: lung elastic elastance (Est) and its viscoelastic (ΔE) component. *Lower panels*: lung resistive (ΔP1) and viscoelastic/inhomogeneous (ΔP2). Values are mean and SD of 10 measurments in each animal (8–9 mice per group). Boxes represent the 25–75% interquartile range, horizontal line is the median, and the whiskers encompass data range. *Values significantly different from C ($p < 0.05$).

last pellet (plasma membrane enriched fraction) was resuspended in 300 μL buffer 1. In order to obtain the microssomal enriched fraction, the supernatant of the first centrifugation was centrifuged at 100,000 × *g* for 35 min at 4 °C. The pellet was washed 2x and resuspended in 0.5 mL buffer 1. The fractions were incubated in 150 mM sodium phosphate buffer (pH 7.4), containing SOD (100 U/mL; Sigma-Aldrich, St. Louis, MO, USA), horseradish peroxidase (0.5 U/mL; Roche, Indianapolis, IN, USA), Amplex red (50 μM; Molecular Probes, Eugene, OR, USA), and 1 mM EGTA in the presence of 1 mM NADPH. To evaluate DUOX activity, plasma membrane enriched fraction was incubated with or without 1 mM $CaCl_2$. The fluorescence was immediately measured in a microplate reader (Victor X4; PerkinElmer, Norwalk, CT, USA) at 30 °C, using excitation at 530 nm and emission at 595 nm (Frankenfeld et al., 2014).

The specific enzymatic activity was expressed as nmol H_2O_2 per hour and milligram of protein (nmol/h/mg). Protein concentration was determined by the Bradford assay (Bradford, 1976).

2.6. Antioxidant enzymes' activity

The right lungs from the same nine animals used for cytokine determination were homogenized in 5 mM Tris-HCl buffer (pH 7.4), containing 0.9% NaCl (w/v) and 1 mM EDTA, followed by centrifugation at 750 × *g* for 10 min at 4 °C. The supernatant aliquots were stored at −80 °C. Catalase activity was assayed following the method of Aebi (1984) and was expressed as units per milligram of protein (U/mg) (Aebi, 1984). Glutathione peroxidase (GPx) activity was assayed by following NADPH oxidation at 340 nm in the presence of an excess of glutathione reductase, reduced glutathione and tert-butyl hydroperoxide as substrates (Flohé and Günzler, 1984), and expressed as nmol of oxidized NADPH per milligram of protein (nmol/mg). Total superoxide dismutase activity was determined by reduction of cytochrome C at 550 nm (Crapo et al., 1977).

2.7. Oxidative stress biomarkers

Total number of tissue thiol groups was determined by spectrophotometry in accordance to Sedlak and Lindsay (1968). Briefly, lung homogenates were incubated in the presence or absence of 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB: 150 μL of 200 mM TRIS + 20 mM EDTA at 8.2 pH; 150 μg protein; in methanol up to 1 mL) at 25 °C for 15 min. Samples were centrifuged at 3000 × *g* for 15 min. The thiol content was determined in the supernatant at 412 nm, and expressed as nanomols of reduced DTNB/mg of protein. Finally, protein carbonyl was determined in lung homogenate by protein reaction with 2,4-dinitrophenylhydrazine (DNPH), using 100 mM phosphate buffer + 5 mM EDTA, HCl-acethone (1:300), tricloroacetic acid, 2 M HCl and 10 mM DNPH, at 405 nm (Fortunato et al., 2013).

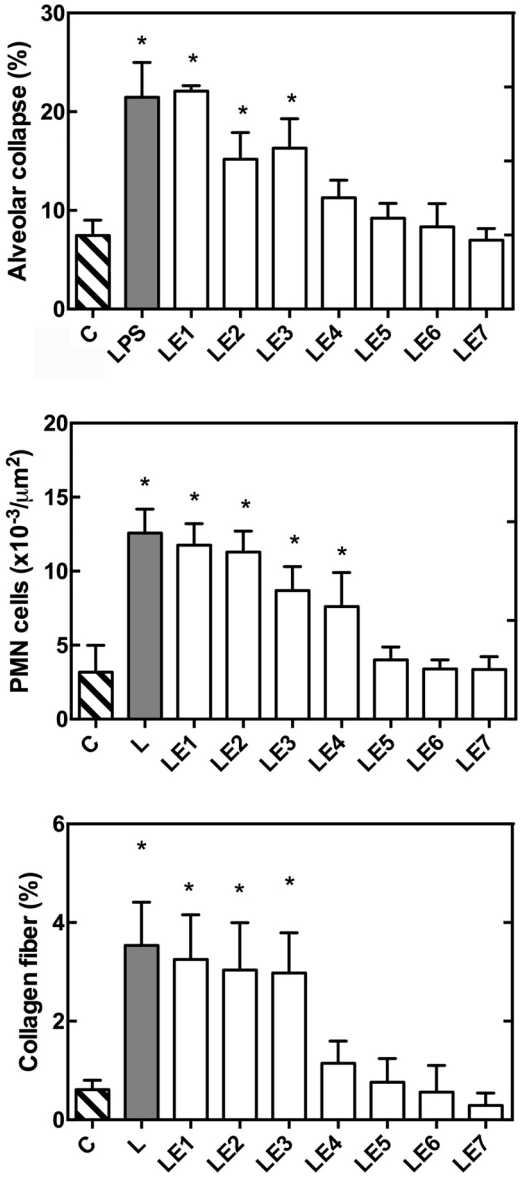

Fig. 2. Eugenol reduced LPS-induced lung histological changes in a dose-dependent manner. Percent of alveolar collapse in relation to the total number of alveoli per field (*upper panel*), number of polymorphonuclear (PMN) cells per lung tissue area (*middle panel*), and percent of lung tissue area occupied by collagen fiber (*lower panel*) in mice 24 h after saline (C) or lipopolysaccharide (10 μg, L) intratracheal instillation, with and without treatment with increasing doses of eugenol (15, 50, 100, 150, 500, 1000, and 1500 mg/kg; groups LE1-7, respectively). Values are mean + SD of 7–9 mice per group (10 non-coincident fields per animal). Columns are means + SD. *Values significantly different from C ($p < 0.05$).

2.8. Statistical analysis

GraphPad Prism 5 statistical package (GraphPad Software, San Diego, CA, USA) was used. Data normality and homogeneity of variances were tested (Kolmogorov-Smirnov test with Lilliefors' correction and Levene median test, respectively). One-way ANOVA was used, followed by Tukey test, when both conditions were attended; otherwise, Kruskal-Wallis ANOVA was applied, followed by Dunn test. Percentage values underwent arcsine transformation prior to statistical tests. Significance level was set at 5%.

3. Results

Firstly, an oral dose-response curve was obtained in order to verify whether the respiratory improvement previously observed in eugenol-treated LPS-induced ALI could be reproduced. LPS increased lung elastic and viscoelastic components in relation to control, as previously reported (Fig. 1). Alveolar collapse, neutrophil infiltration, and collagen fiber deposition in lung parenchyma were also observed, supporting model reproducibility (Fig. 2). Eugenol treatment at 150 mg/kg by gavage reduced lung elastance back to control values, as well as alveolar collapse and collagen fiber deposition in lung parenchyma. Since eugenol at 150 mg/kg by gavage reproduced previously observed functional and morphological improvement, this dose was used in the subsequent experiments.

We have previously reported that eugenol treatment improved LPS-induced lung changes through its anti-inflammatory effect, reducing TNF-α production and NF-κB activation. The present results further elucidate the mechanisms of eugenol beneficial action on LPS-induced ALI. Eugenol treatment reduced TNF-α levels in lung homogenate, supporting our previous observation of eugenol-induced reduction in TNF-α level in BAL fluid. Additionally, it also reduced IL-1β and IL-6 levels in lung homogenate to values similar to those observed in control mice (Fig. 3).

Regarding oxidative stress, LPS increased NOX activity in lung tissue, both in plasma membrane and microssomal fractions. No significant change was observed, however, in DUOX activity (Fig. 4). LPS exposure increased catalase, SOD, and GPx activities in lung homogenate (Fig. 5). LPS also augmented protein oxidation, as shown by increased carbonyl levels in L group compared with control, but no change was observed in total reduced thiol content (Fig. 6). LPS-induced changes in NOX and antioxidant enzymes activities, as well as protein carbonyl levels were significantly reduced by eugenol treatment (Figs. 4–6).

4. Discussion

Our results demonstrated, in an *in vivo* model of LPS-induced ALI, that eugenol treatment avoided lung functional and morphological changes (Figs. 1 and 2), through the modulation of both lung inflammation (Fig. 3) and oxidative stress elicited by LPS (Figs. 4–6).

We have previously reported the anti-inflammatory action of eugenol in LPS-induced ALI (Magalhães et al., 2010). The present results reproduced the observed benefits after a more convenient oral treatment, and further clarified the mechanisms therein involved. Additionally, we showed for the first time the therapeutic anti-oxidative action of eugenol *in vivo*, stressing its role in LPS-induced lung oxidative injury.

LPS exposure yields an acute inflammatory lung response similar to that observed in acute respiratory distress syndrome (Faffe et al., 2000), mainly orchestrated by TNF-α secretion and NF-κB activation (Zhang et al., 2001; Covert et al., 2005; Thorley et al., 2007). The LPS-induced ALI model is characterized by respiratory mechanical changes associated with alveolar collapse, collagen deposition and neutrophil infiltration in lung parenchyma, as well as TNF-α release into brochoalveolar lavage fluid (BALF) and NF-κB activation (Menezes et al., 2005; Santos et al., 2006; Magalhães et al., 2010). It is noteworthy that in the present study, eugenol treatment was applied 6 h after LPS exposure, when the inflammatory cascade was already initiated (Faffe et al., 2000; Menezes et al., 2005; Santos et al., 2006), underlying its therapeutic potential in LPS-induced ALI.

After oral administration, eugenol is readily absorbed and distributed to different organs (Guénette et al., 2007), being almost totally eliminated after its metabolization (Fischer et al., 1990). The dose-response curve showed a dose-dependent effect of eugenol. Oral treatment with 150 mg/kg reproduced the beneficial effects of eugenol when administered intraperitoneally (Magalhães et al., 2010),

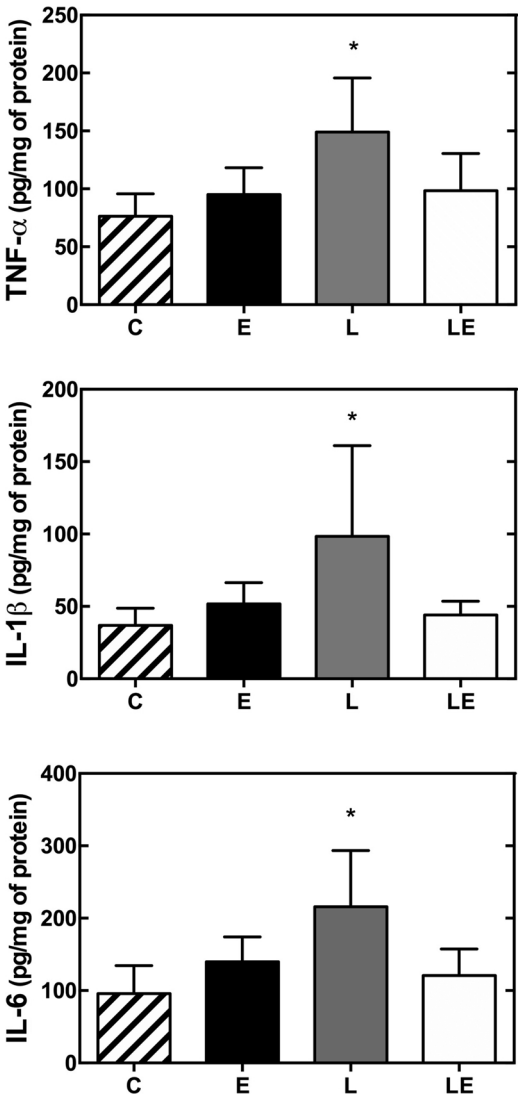

Fig. 3. Eugenol reduced LPS-induced inflammatory cytokines release. Levels of TNF-α (*upper panel*), IL-1β (*middle*), and IL-6 (*lower panel*) in lung homogenate determined by ELISA 24 h after saline (C), eugenol (E) or lipopolysaccharide (10 μg, L) intratracheal instillation, with and without treatment with eugenol (150 mg/kg; groups LE and L, respectively). Values are mean + SD of 8–9 mice per group. *Values significantly different from all other groups ($p < 0.05$).

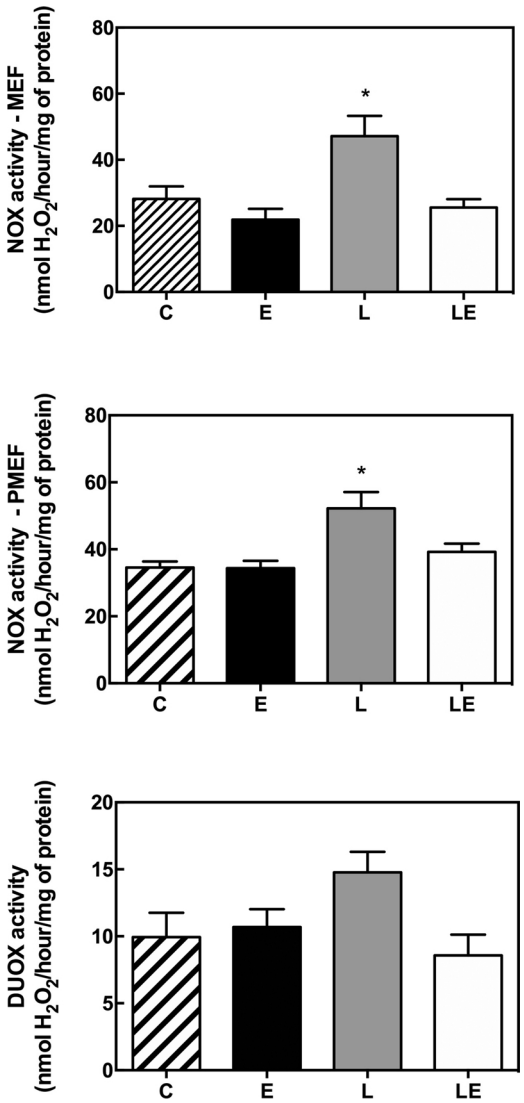

Fig. 4. Eugenol treatment reduced LPS-induced changes in NADPH oxidase activity. NADPH oxidase activity in lung membrane enriched fractions 24 h after intratracheal instillation of either saline (C), eugenol (E), or lipopolysaccharide (10 μg, L) with and without treatment with eugenol (150 mg/kg; groups LE and L, respectively). *Upper and middle panels*: NOX activity in microsomal (MEF) and plasma membrane (PMEF) enriched fractions, respectively. *Lower panel*: DUOX activity in membrane enriched fraction. Values are mean + SD of 6 mice per group. *Values significantly different from all other groups ($p < 0.05$).

improving LPS-induced changes in lung mechanics and neutrophil infiltration in lung parenchyma. The anti-inflammatory action of eugenol in this model can be explained by its effect on TNF-α levels and NF-κB activation (Magalhães et al., 2010), as well as on inflammatory cytokine release, as supported by IL-1β and IL-6 levels observed in the present study. These results reproduce *in vivo* the preventive effect of eugenol on NF-κB activation, inflammatory cytokine release, and COX-2 expression observed on LPS-stimulated macrophages in culture (Murakami et al., 2003, 2005). Our data also support *in vivo* previous observation in human macrophages, demonstrating that eugenol down-regulates IL-1 protein level and TNF-α induced by LPS (Lee et al., 2007). Furthermore, the eugenol oral dose-response curve agrees with the observation that phenolic antioxidants inhibit TNF-α production in a complete, long lasting, and dose dependent way (Ma and Kinneer, 2002).

It is well known that pro-inflammatory signaling promotes the accumulation of ROS. Moreover, NADPH oxidases seem to be the main source of ROS after lung exposure to LPS (Carnesecchi et al., 2012). In the present study, eugenol abolished the increase of NOX activity induced by LPS. Thus, it seems that the anti-inflammatory action of eugenol was crucial to prevent the induction of NOX enzymes by LPS. Our data concerning antioxidant enzymes activity showed that SOD, GPx, and catalase were increased in the lung of the LPS-treated group, which was abolished by eugenol treatment. Redox-sensitive transcription factors, such as NRF2, NF-κB, and AP-1, are crucial to activate antioxidant defense in oxidative stress conditions (Hecht et al., 2016). NOX activity was decreased by eugenol, reducing ROS availability, and consequently decreasing the activation of the signaling pathways mentioned above. It is important to note that DUOX enzymes are crucial to innate immune response in the airways, because of that we expected a greater activity after LPS-exposure. In fact, we can not rule out a role for DUOX in LPS-induced ROS generation. Although the DUOX activity after LPS did not show a significant difference, we observed an increase in LPS-treated group which was also reduced to control values by eugenol (Bernard et al., 2014). Furthermore, eugenol and other

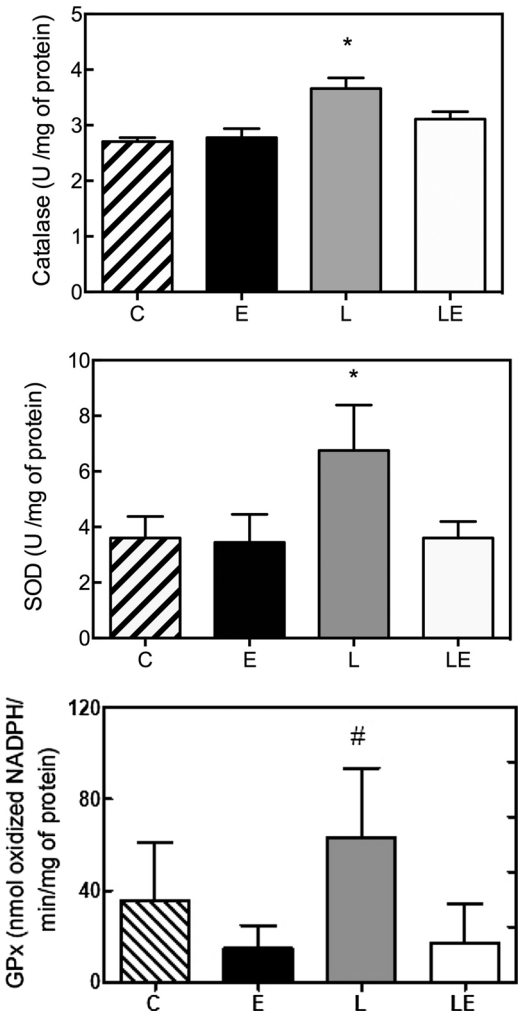

Fig. 5. Eugenol treatment reduced LPS-induced activation of oxidative enzymes. Catalase (*upper panel*), superoxide dismutase (SOD, *middle*), and glutathione peroxidase (GPx, *lower panel*) activities in lung homogenate 24 h after intratracheal instillation of either saline (C), eugenol (E), or lipopolysaccharide (10 μg, L) with and without treatment with eugenol (150 mg/kg; groups LE and L, respectively). Values are mean + SD of 8–9 mice per group. *Values significantly different from all other groups, #values significantly different from E and LE ($p < 0.05$).

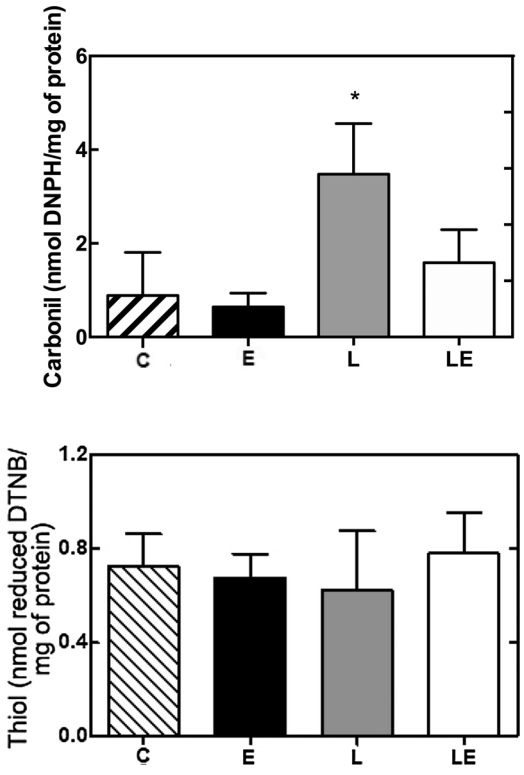

Fig. 6. Eugenol treatment reduced LPS-induced protein oxidation. Total number of carbonyl protein (*upper panel*) and thiol groups (*lower panel*) in lung homogenate 24 h after intratracheal instillation of either saline (C), eugenol (E), or lipopolysaccharide (10 μg, L) with and without treatment with eugenol (150 mg/kg; groups LE and L, respectively). DNPH: 2,4-dinitrophenylhydrazine. DTNB: 5,5′-dithiobis(2-nitrobenzoic acid). Values are mean + SD of 8–9 mice per group. *Values significantly different from all other groups ($p < 0.05$).

phenolic compounds have antioxidative effects, due to their capacity to interact and detoxify ROS (Ma and Kinneer, 2002; Murakami et al., 2003, 2005). Finally, eugenol treatment avoided protein oxidation, as evidenced by carbonyl levels in LE group similar to control group's, corroborating the results of NOX and antioxidant activities.

Several studies indicate that NF-κB activation in the lungs is partially controlled by ROS, thus, modulating LPS-induced cytokine release (Asehnoune et al., 2004; Park et al., 2004; Ngkelo et al., 2012). Furthermore, since SOD, CAT, and GPx activities were not increased after eugenol treatment, our data suggest that the anti-oxidative action of eugenol in LPS-stimulated lungs is mainly mediated by modulation of cytokine release through NOX4 and ROS sensitive mechanisms.

A preventive anti-oxidative effect of eugenol was previously reported in LPS-induced ALI when administered pre-LPS challenge (Huang et al., 2015). Although a small eugenol dose (5 and 10 mg/kg) showed some effectiveness, it was not able to completely avoid inflammation or oxidative stress, while no functional information was available. Eugenol administration prior to LPS challenge could avoid neutrophil infiltration, thus reducing inflammation and oxidation. In the clinical scenario, however, the LPS-induced changes are already in progress. Furthermore, many anti-inflammatory/oxidative effects observed experimentally are, in fact, not able to affect lung function, reducing its translational relevance. Thus, the question of whether eugenol could stop or even revert an ongoing injury still remained. Our work supports a therapeutic potential for eugenol, reporting its effectiveness after the full establishment of LPS-induced lung changes, as well as clarifying the mechanisms involved. The beneficial effects of eugenol were strengthened by the observation that under higher doses (150 mg/kg) it improved lung function. Our data yields a better understanding of eugenol action and its mechanisms.

In conclusion, taken together, our results support a beneficial role for eugenol in LPS-induced ALI through both anti-inflammatory and anti-oxidative actions.

Conflict of interest

The authors declare that they have no conflict of interest.

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