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ORIGINAL ARTICLE

Low-power laser alters mRNA levels from DNA repair genes in acute lung injury induced by sepsis in Wistar rats

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Abstract

Acute lung injury (ALI) is defined as respiratory failure syndrome, in which the pathogenesis could occur from sepsis making it a life-threatening disease by uncontrolled hyperinflammatory responses. A possible treatment for ALI is the use of low-power infrared lasers (LPIL), whose therapeutical effects depend on wavelength, power, fluence, and emission mode. The evaluation mRNA levels of repair gene related to oxidative damage after exposure to LPIL could provide important information about the modulation of genes as treatment for ALI. Thus, the aim of this study was to evaluate the mRNA levels from *OGG1*, *APEX1*, *ERCC2*, and *ERCC1* genes in lung tissue from Wistar rats affected by ALI and after exposure to LPIL (808 nm; 100 mW). Adult male Wistar rats ($n = 30$) were randomized into six groups ($n = 5$, for each group): control, 10 J/cm^2 (2 J), 20 J/cm^2 (5 J), ALI, ALI + LPIL 10 J/cm^2 and ALI + LPIL 20 J/cm^2. ALI was induced by intraperitoneal *E. coli* lipopolysaccharide injection (10 mg/kg). Lungs were removed, and samples were withdrawn for total RNA extraction, cDNA synthesis, and mRNA levels were evaluated by RT-qPCR. Data normality was verified by Kolmogorov-Smirnov, comparisons among groups were by Student's *t* test, Mann-Whitney test, one-way ANOVA, Kruskal-Wallis followed by post-tests. Data showed that OGG1 (0.39 ± 0.10), ERCC2 (0.67 ± 0.24), and ERCC1 (0.60 ± 0.19) mRNA levels are reduced in ALI group when compared with the control group (1.00 ± 0.07, 1.03 ± 0.25, 1.01 ± 0.16, respectively) and, after LPIL, mRNA relative levels from DNA repair genes are altered when compared to non-exposed ALI group. Our research shows that ALI alter mRNA levels from genes related to base and nucleotide excision repair genes, suggesting that DNA repair is part of cell response to sepsis, and that photobiomodulation could modulate the mRNA levels from these genes in lung tissue.

Keywords Acute lung injury · DNA repair · Low-power laser · Sepsis · Wistar rats

Introduction

Acute lung injury (ALI) is defined, according to European American Consensus Conference [1, 2], as an acute respiratory failure syndrome characterized by bilateral pulmonary infiltrate in a chest radiography, compatible with pulmonary edema. Presenting severe hypoxemia, the ratio of arterial oxygen to the fraction of inspired oxygen (PaO_2/FiO_2) must

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be 300 mmHg or less, regardless of the level of positive end-expiratory pressure (PEEP), besides pulmonary artery occlusion pressure 18 mmHg or less absence of clinical or echocardiographic signs of left atrial hypertension [1, 2].

Acute respiratory distress syndrome (ARDS) has a similar definition to ALI, but ARDS is the most severe form of ALI, when the ratio of arterial oxygen to fraction of inspired oxygen is equal or lower than 200 mmHg [1, 2], life-threatening disease characterized by uncontrolled hyper inflammatory responses in lungs [3].

The Berlin definition and mortality rate, shown in Table 1, classifies ARDS as mild, moderate, and severe. By this definition, ARDS presenting respiratory failure not fully explained by cardiac failure or fluid overload, bilateral opacities not fully explained by effusions, lung collapse, or nodules. ARDS is well characterized by increased permeability as reflected by alveolar edema due to epithelial and endothelial cell damage and neutrophil infiltration. The term acute lung injury was maintained for experimental animal models [4].

Epidemiology of ARDS/ALI shows an average of about 41 cases per 100,000 people per year in the USA [5, 6] with clear seasonal variations, being more frequent in winter. Epidemiological surveys report 190,000 patients hospitalized annually, bringing a huge expense to health system [7, 8]. ALI incidence increases with age, affecting 306 persons per 100,000 people per year, in individuals ranging of 75 to 84 years [5]. Mortality is high reaching up to 45% [5].

Studies showed the pathogenesis of ARDS/ALI development at cellular and molecular levels, in which the progression of pulmonary ARDS/ALI toward lung injury occurs at alveolar epithelium, whereas in extrapulmonary ARDS/ALI, the target is the microvascular endothelium [9]. In the latter, injury could occur after sepsis, pancreatitis and septic shock [10]. However, ARDS/ALI development of the disease is relatively uniform with diffuse inflammation in lung tissue [9]. This inflammatory process establishes alveolar and interstitial edema progressing to lung fibrosis [9, 10]. Inflammatory cells, mainly neutrophils, persist with release of mediators that prevent the resolution of inflammation [11]. Inflammatory mediators trigger systemic inflammation, which could affect other organs, being the main cause of death of ARDS/ALI patients [11, 12]. Inflammatory response to an infection, occurring in sepsis, is initiated by the binding of microbial products at specific receptors on the surface of macrophages, such as TLR2 and TLR4 [13, 14]. For instance, these microbial products could be a bacterial cell wall components, as lipopolysaccharides from Gram-negative bacteria. Inflammatory response increases production of reactive oxygen species (ROS), such as superoxide anion ($O_2^{\bullet}$) and nitric oxide (NO), in endothelial and epithelial cells [15]. The excessive production of ROS occurs in hypercapnia, acidosis, and pulmonary hypertension, which threaten the patients' life [3, 11] and an accurate therapy without prejudice to injured tissue is required. There is no specific treatment for ARDS/ALI.

Studies have shown that oxidative stress in patients with sepsis leads to plasma reduction, reduction of total antioxidant capacity, and elevated levels of malondialdehyde and 4-hydroxynonenal [16]. Other studies indicate a marker for oxidative stress in ARDS, glutathioneperoxidase (GPx) that correlates with PaO_2/FiO_2, and the oxidation index initially and in the progression of the disease correlates with the PEEP used in treatments [17]. Sarkele and coworkers (2014) found that ARDS/ALI patients had higher concentrations of GSSG (the oxidized form of glutathione) compared to the control group, despite elevated levels of F2-isoprostane as a marker for lipid peroxidation, thus indicating oxidative stress in patients with ARDS/ALI [18].

A possible treatment for ARDS/ALI is the use of low-power lasers in the infrared range of the electromagnetic spectrum, which have been used successfully for resolution of inflammatory processes [3, 19]. Despite these radiation beams are collimated, monochromatic, and have high energy densities [20], low-power laser-induced therapeutic effects are obtained at low fluences, energies (or doses), and they depend on wavelength, frequency, power, and emission mode [21, 22].

Recent tissue studies demonstrate penetration of low-power infrared that generates a long narrow volume of coherent light can penetrate deeper into tissues [23, 24]. A study shows that 808 nm light penetrates as much as 54% deeper than 980 nm [25]. Byrnes and coworkers reinforce that near infrared light in the range 770–850 nm penetrates deeper through rat tissues than red light or longer infrared wavelengths, up to 1200 nm [26]. Models show that the penetration into any biological tissue when applied percutaneously reaches between 2 and 5 cm, depending on the tissue layers and the metabolic state of the patient [27, 28].

The action of low-power lasers is based on the absorption of light by the cells, in which they modulate biochemical

Table 1 Berlin categories definition for ARDS/ALI

Categories	Oxygenation	Mortality (%)
Mild	200 mmHg < PaO_2/FIO_2 ≤ 300 mmHg with PEEP or CPAP ≥ 5 cm H_2O	27
Moderate	100 mmHg < PaO_2/FIO_2 ≤ 200 mmHg with PEEP or CPAP ≥ 5 cm H_2O	32
Severe	PaO_2/FIO_2 ≤ 100 mmHg with PEEP ≥5 cm H_2O	45

PEEP, positive end-expiratory pressure; CPAP, continuous positive airway pressure; PaO_2, partial pressure of arterial oxygen; FIO_2, fraction of inspired oxygen

reactions and stimulate mitochondrial respiration [20]. These effects could accelerate the synthesis of DNA, RNA, and regulatory proteins of cell cycle, promoting proliferation [22], as well increasing the production of reactive oxygen species and reactive nitrogen species. These free radicals could participate as second messengers in cell signaling pathways [29]. On the other hand, free radicals from low-power lasers could react with DNA causing lesions at sublethal level [30] and modulate mRNA levels from DNA repair gene [31].

Patients suffering ARDS/ALI could present lung cells on oxidative stress and DNA lesions in pulmonary and adjacent cells [32]. Once the DNA molecule is damaged, a set of enzymatic mechanisms are trigged to preserve genetic code integrity and cellular homeostasis [33]. These enzymatic mechanisms include the base and the nucleotide excision repair pathways.

Base excision repair (BER) generally acts on oxidative bases and few bulky adducts, through a specific enzymatic action protein, such as 8-oxoguanine DNA glycosylase (OGG1), responsible for removing oxidized guanines [34], followed by cleavage of the sugar-phosphate chain by an apurinic/apyrimidinic endonuclease, such as APEX-1 [35]. This is the short-patch BER pathway and results in the replacement of the apurinic/apyrimidinic site by incorporation of a single nucleotide. In contrast, the BER long-patch pathway produces a repair of at least two nucleotides [26]. Some DNA glycosylases are classified as monofunctional, that is, they only remove the damaged bases [34, 36]. Others are classified as bifunctional, since in addition to removing the damaged base, they also cleave the DNA molecule at the lesion site creating an apurinic/apirimidine site (AP site) and another nitrogenous base can be inserted in the next phase [34, 35]. If the DNA glycosylase is monofunctional, another endonuclease cleaves the DNA molecule at the site of the removed base, such as apurinic/apyrimidinic endonuclease 1 (APEX-1) [35]. In the next step, the insertion of a new nitrogen base through the DNA polymerase β occurs, and finally, the DNA tape is sealed by the action of DNA ligase [37].

Nucleotide excision repair (NER) is involved in finding and removing DNA lesions, which cause distortions in DNA double helix [38]. Recently, NER was also demonstrated acts on oxidative damages in DNA [39]. NER is dependent on RNA polymerase II action when repair is coupled to transcription, carrying out rapid repair of actively transcribed genes [40], but it can be independent of RNA polymerase II action by removing the lesions located anywhere in the genome [41]. NER operates with other enzymes, as the ERCC2 (excision repair cross-complementing group 2) and complex ERCC1 (excision repair cross-complementing group 1) [42, 43]. ERCC2 gene product is associated with DNA unwinding when its product (protein XPD) is expressed [43]. XPD proteins act in the process as temporary helicase rolling out double helix to find DNA lesions caused by free radicals [44] with subsequent lesion removal [45]. Lesion removal is carried out by a heterodimer constituted by ERCC1/XPF proteins [43]. This heterodimer executes a 5′ incision on the DNA strand at the DNA lesion site after all other excision steps have been performed and, therefore, have been completed [46]. After that, DNA repair synthesis is performed by DNA polymerase using the opposite undamaged DNA strand as template [47], and connection with the DNA strand is carried out by DNA ligase [48].

The evaluation of mRNA levels from repair genes related to oxidative damage after exposure to low-power infrared laser could provide important information about the modulation of genes as treatment for sepsis-affected lung tissue. We hypothesize that low-power infrared laser modulates the mRNA levels from DNA repair genes in the acute lung injury. Thus, the aim of this study was to evaluate the mRNA levels from OGG1, APEX1, ERCC2, and ERCC1 genes in lung tissue from Wistar rats affected by acute lung injury in response to sepsis and after exposure to low-power infrared laser at different fluences. Acute lung injury is understood by ARDS/ALI.

Materials and methods

Animals

Experiments were conducted in accordance with the Ethics Committee in Animal Experiments of Universidade Federal de Juiz de Fora, Minas Gerais, Brazil, protocol number 012/2016. Over the week, prior to the experiment procedure, groups of five animals were housed in clear plastic cages with stainless steel wire lids (Beira-Mar Industria e Comércio, St. Antônio, São Paulo, Brazil) and pinewood shavings (*Pinus elliottii*, MF Rural, Alto Cafezal, São Paulo, Brazil) as bedding. Additionally, the rats were kept in an vivarium of the Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro, Brazil with controlled environmental conditions (12-h light/12-h dark cycle, temperature 22 °C) on closed ventilated shelves. Animals received rat chow pellets (Nuvital Nutrientes, Colombo, Paraná, Brazil) and water (reservoirs of Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil) ad libitum.

Low-power laser

A therapeutic low-power infrared (808 nm) laser (Photon Lase III, AsGaAl), purchased from D.M.C. Equipamentos Ltda (São Paulo, Brazil), in continuous wave emission mode was used in this study. Laser physical parameters are in Table 2.

Table 2 Laser physical parameters

Wavelength (nm)	808
Diode laser	AsGaAl
Power (mW)	100
Spot size (cm^2)	0.028
Power density (W/cm2)	3.571
Energy per point (J)	2 and 5
Energy density (J/cm^2)	10 and 20
Time per point (s)	2 and 5
Number of points per lung	4
Application technique	Punctual by skin contact
Number of treatment sessions	One session

Reagents

Escherichia coli lipopolysaccharide (LPS) serotype 055:B5 was from Sigma-Aldrich (St. Louis, MO, USA), TRIzol® reagent was from Invitrogen (Carlsbad, CA, USA), isopropanol, chloroform, and ethanol were from Merck (Darmstadt, Germany), diethyl pyrocarbonate (DEPC) was from Sigma (St. Louis, MO, USA), NaCl (sodium chloride) was purchased from Vetec (Duque de Caxias, RJ, Brazil) and Entellan (Merck, Darmstadt, Germany).

Experimental procedure

Animals ($n = 30$) were randomly assigned to six main groups of five animals each: (1) C (Control) were animals treated with saline solution (SS 0.9% NaCl); (2) L10 (laser 10 J/cm^2) were animals treated with SS and exposed to infrared laser at 10 J/cm^2; (3) L20 (laser 20 J/cm^2) were animals treated with SS and exposed to infrared laser at 20 J/cm^2; (4) ALI (acute lung injury) were animals treated with LPS (10 mg/kg) dissolved in SS; (5) ALI-L10 were animals treated with LPS (10 mg/kg) dissolved in SS and, after 4 h, were exposed to infrared laser at 10 J/cm^2; (6) ALI-L20 were animals treated with LPS (10 mg/kg) dissolved in SS, and after 4 h, were exposed to infrared laser at 20 J/cm^2, as in Fig. 1.

The irradiation occurred after sedation with intraperitoneal bolus of ketamine (80 mg/kg—Dopalen, Vetbrands, Miramar, FL, USA) and xylazine (8 mg/kg—Sedazine, Fort Dodge, IA, USA), dorsal decubitus position and tricotomy of the thoracic region of the Wistar rats, determined four points between the ribs along each lung (Fig. 2). After 24 h of LPS-induced sepsis or after the last exposure to infrared laser, animals were euthanized by anesthetic overdose and thoracotomy was performed. Trachea was clamped and lungs were removed for further analysis.

Processing and qualitative histological analysis of lung tissue

After euthanasia, samples from left lung tissues were collected and fixed (4% buffered formaldehyde, 0.01 M, pH 7.4—Labsynth, Diadema, SP, Brazil) and processed for paraffin embedding. Four-micrometer thick sagittal slices (Lupetec, São Carlos, SP, Brazil and Labsynth, Diadema, SP, Brazil) were done and stained with hematoxylin–eosin (Labsynth, Diadema, SP, Brazil). The preparations were air dried and mounted in Entellan. Qualitative histological analyses were performed using a conventional light Olympus microscope (BX53F), equipped with the U-PlanFL N 4/0.13, 10/0.30, 40/0.75 and 100/0.85 objectives. The images were captured with an Olympus DP73 camera, using the cellSens Imaging software version 5.1, Olympus, USA.

Evaluation of mRNA relative levels of DNA repair genes

Procedure

After euthanasia, samples (50 up to 100 mg) from right lung tissue were collected and transferred to microcentrifuge (Heraeus Fresco 17 Microcentriguge – Thermo Fisher Scientific, USA) flex tubes with TRIzol® reagent (Invitrogen—Carlsbad, CA, USA) for total RNA extraction by standard procedure.

Total RNA extraction

Tissue samples were crushed into microcentrifuge flex tubes with TRIzol® reagent and centrifuged (12,000×*g*, 4 °C, 10 min—Heraeus Fresco 17 Microcentriguge, Thermo Fisher Scientific, USA). Supernatants were transferred to other tubes, chloroform (Merck, Darmstadt, Germany) was added, mixtures were centrifuged (12,000×*g*, 4 °C, 15 min), aqueous phases were transferred to other tubes, and isopropanol (Merck, Darmstadt, Germany) was added. After incubation (room temperature, 15 min), mixtures were centrifuged (12,000×*g*, 4 °C, 10 min), supernatants were discarded, and precipitate was washed with ethanol-DEPC (80% ethanol from Merck, Darmstadt, Germany and DEPC 0.1% from Sigma, St. Louis, MO, USA) solution added and centrifuged. Supernatants were withdrawn and total RNA was reconstituted in water-DEPC (0.1% from Sigma, St. Louis, MO, USA) solution and stored (− 80 °C).

Complementary DNA synthesis

RNA concentration and purity were determined on a spectrophotometer (Thermo Scientific Nano-Drop 2000 Spectrophotometer, Waltham, MA, USA) by calculating the

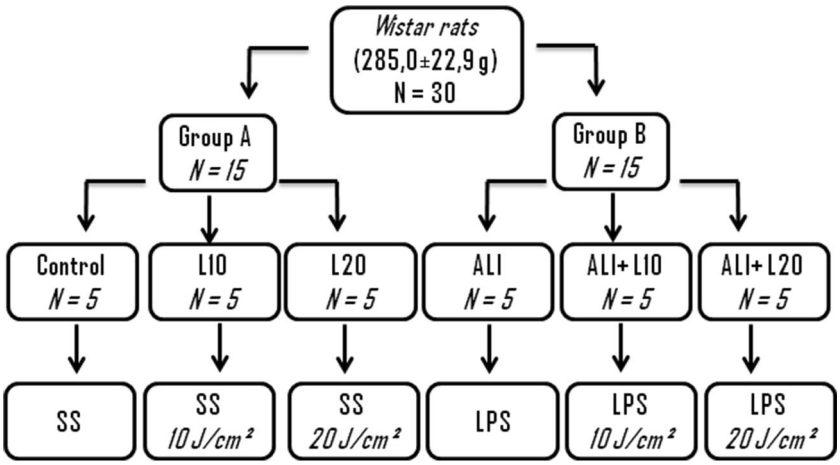

Fig. 1 Diagrammatic representation to demonstrate the summary number and treatments for animal groups

optical density ratio at a 260/280 nm wavelength ratio. Complementary DNA (cDNA) synthesis was carried out using a two-step cDNA synthesis kit (Promega, Durham, NC, USA). Four micrograms of RNA were reverse transcribed into cDNA using GoScript™ reverse transcriptase (Promega, Durham, NC, USA), according to the manufacturer's protocol, using a total 20 μL reaction. Real-time quantitative polymerase chain reaction (Applied Biosystems 7500 RT-qPCR, Thermo Fisher Scientific, Waltham, MA, USA) was performed using 5 μL of GoTaq qPCR Master Mix (Promega, Durham, NC, USA) for a final volume of 10 μL volume containing 50 ng of cDNA. To determine the initial relative of cDNA quantity, samples were amplified with Apurinic/Apyrimidinic Endodeoxyribonuclease 1 (APEX1), 8-Oxoguanine glycosylase (OGG1), excision repair cross-complementation group 1 (ERCC1), excision repair cross-complementation group 2 (ERCC2) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers (IDT, Coralville, IA, USA). Reactions, in duplicate for each sample, were run on an Applied Biosystems 7500 RT-qPCR machine (Applied Biosystems, Thermo Fischer Scientific, Waltham, MA, USA). The PCR were initially denatured at 94 °C for 10 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s (except OGG1, whose annealing step was 67 °C for 30 s), and an extension period at 72 °C for 30 s. Melt curve analyses were performed for all genes and PCR product specificity were confirmed by the presence of a single peak (Applied Biosystems 7500 Real-Time PCR Software, Thermo Fischer Scientific, Waltham, MA, USA). Relative expression was normalized by reference gene levels (GAPDH), using ALI rats from control group. Duplicate Ct values were analyzed in Microsoft Excel software (Microsoft, USA) using the comparative Ct ($2-\Delta\Delta CT$) method [49].

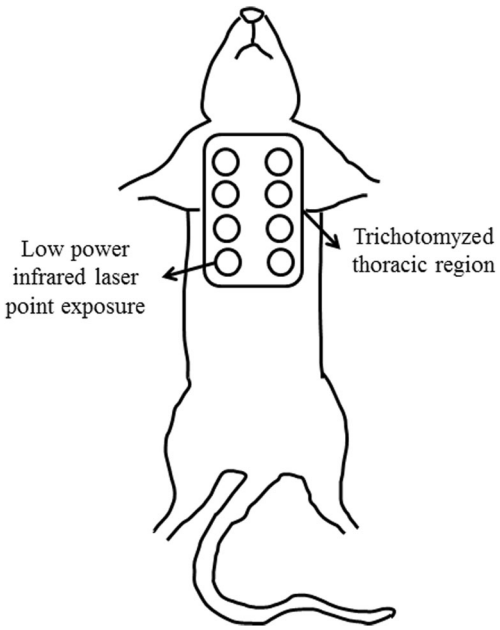

Fig. 2 The protocol was designed to irradiate punctually by positioning the laser pen between the ribs after tricotomy of the animals' thorax and with dorsal decubitus in order to reach to lung

Statistical analysis

Data normality was verified by Kolmogorov-Smirnov test. Data are presented as mean and standard deviation. An unpaired Student's t test and a Mann-Whitney test were performed to compare the acute lung injury group to control group. One-way ANOVA followed by Bonferroni's multiple comparison tests were used to compare parametric data. For nonparametric data, Kruskal-Wallis followed by Dunn's multiple comparison tests. $p < 0.05$ was considered as the less significant level and power of the tests was 75% at least ($\beta < 25$). InStat Graphpad (GraphPad InStat version 5.0 for Windows 8, GraphPad Prism Software, San Diego, CA, USA) and Bioestat (Bioestat version 5.0 for Windows 8, Tefé, AM, Brazil) softwares were used to perform statistical analysis.

Results

Lung morphometry

Pulmonary architecture observed 24 h after acute lung injury (ALI) induction by LPS administration and/or by the last low-power infrared laser exposure (Fig. 3), demonstrated that the ALI induction was efficient through the model of LPS (Fig. 3d). When compared to control group (Fig. 3a), interalveolar septa wall is thickened, presenting alveolar space reduction and intense inflammatory infiltrate with polymorphonuclear cells.

However, photobiomodulation in both fluences used (Fig. 3e, f) reduced interalveolar septum thickening in injured lungs, increasing alveolar space. However, they still present an inflammatory infiltrate when compared to ALI group (Fig. 3d). No significant changes were observed in animals exposed only to low-power infrared laser when compared to control group (Fig. 3b, c).

mRNA relative levels from base and nucleotide excision repair genes in lung tissue from normal and sepsis-induced ALI rats

Figure 4 shows OGG1, APEX1, ERCC2, and ERCC1 mRNA relative levels in lung tissue from rats after sepsis-induced ALI. Data in Fig. 4a show significant ($p < 0.001$) reduction of OGG1 mRNA relative levels in lung tissue from rats after sepsis-induced ALI group when compared to control group. Unlike OGG1, APEX1 mRNA relative levels, in Fig. 4b, shows no significant ($p > 0.05$) alteration in lung tissue from rats after sepsis-induced ALI when compared to control group. Figure 4c shows significant ($p < 0.01$) reduction of ERCC2 mRNA relative levels in lung tissue from rats after sepsis-induced ALI when compared to control group. ERCC1 mRNA relative level was evaluated in lung tissue from rats after sepsis-induced ALI (Fig. 4d). Similar to ERCC2 mRNA, lung tissue from rats after sepsis-induced ALI presented significant ($p < 0.001$) reduction of ERCC1 mRNA levels when compared to control group.

Effects of low-power infrared laser on mRNA relative levels from base excision repair genes in lung tissue from normal and sepsis-induced ALI rats

Figure 5 shows OGG1 mRNA relative levels in lung tissue after sepsis-induced ALI and low-power infrared laser exposure at different fluences (10 and 20 J/cm^2). Data in this figure indicate no significant ($p > 0.05$) alteration of OGG1 mRNA levels in lung tissue from normal rats exposed to infrared laser (L10 and L20) when compared with control group. However, a significant decrease ($p < 0.001$ and $p < 0.01$) of OGG1 mRNA relative levels was obtained in lung tissue from rats after sepsis-induced ALI and exposure to infrared laser at fluences 10 and 20 J/cm^2 ($p < 0.001$ and $p < 0.01$, respectively), when compared to not exposed to low-power infrared laser.

APEX1 mRNA relative levels were evaluated to confirm the effects of photobiomodulation on mRNA levels from base excision repair pathway (Fig. 6). Similar to obtained for OGG1, exposure to infrared laser do not significantly ($p > 0.05$) alter APEX1 mRNA relative levels in lung tissue from normal rats expose to infrared laser (L10 and L20) when compared to control group. Also, no significant ($p > 0.05$) difference in APEX1 mRNA relative levels were obtained in lung tissue from rats after sepsis-induced ALI and exposure to infrared laser at the lower fluence (10 J/cm^2). However, significant decrease ($p < 0.05$) of OGG1 mRNA relative levels was obtained in lung tissue from rats after sepsis-induced ALI and

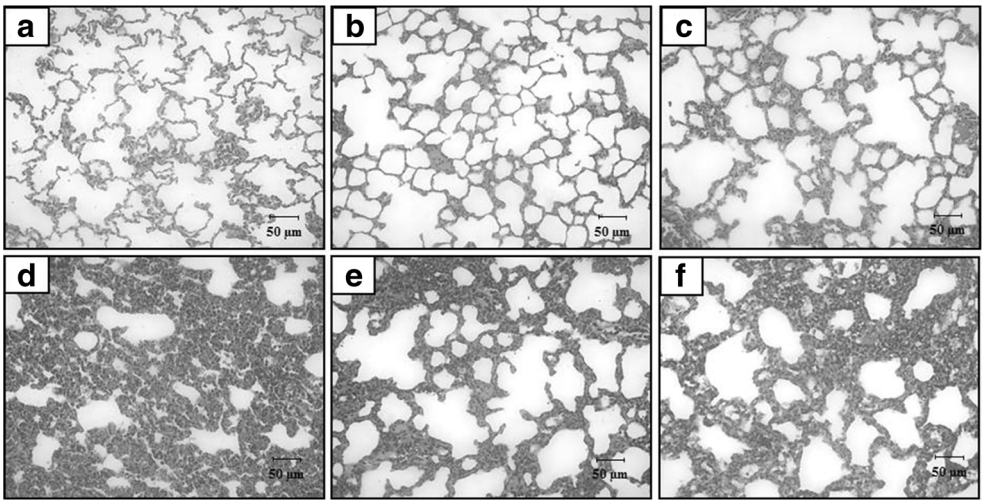

Fig. 3 Representative photomicrographs of lung parenchyma H&E stained. To ip (intraperitoneal), following the groups: 1—*control* (PBS it + saline solution 0.9% ip); 2—*L10*, laser 10 J/cm^2; 3—*L20*, laser 20 J/cm^2; 4—*ALI*, acute lung injury; 5—*ALI-L10*, acute lung injury + laser 10 J/cm^2; 6—*ALI-L20*, acute lung injury + laser 20 J/cm^2. Main figure was captured with a × 40 objective lens (bar = 50 µm)

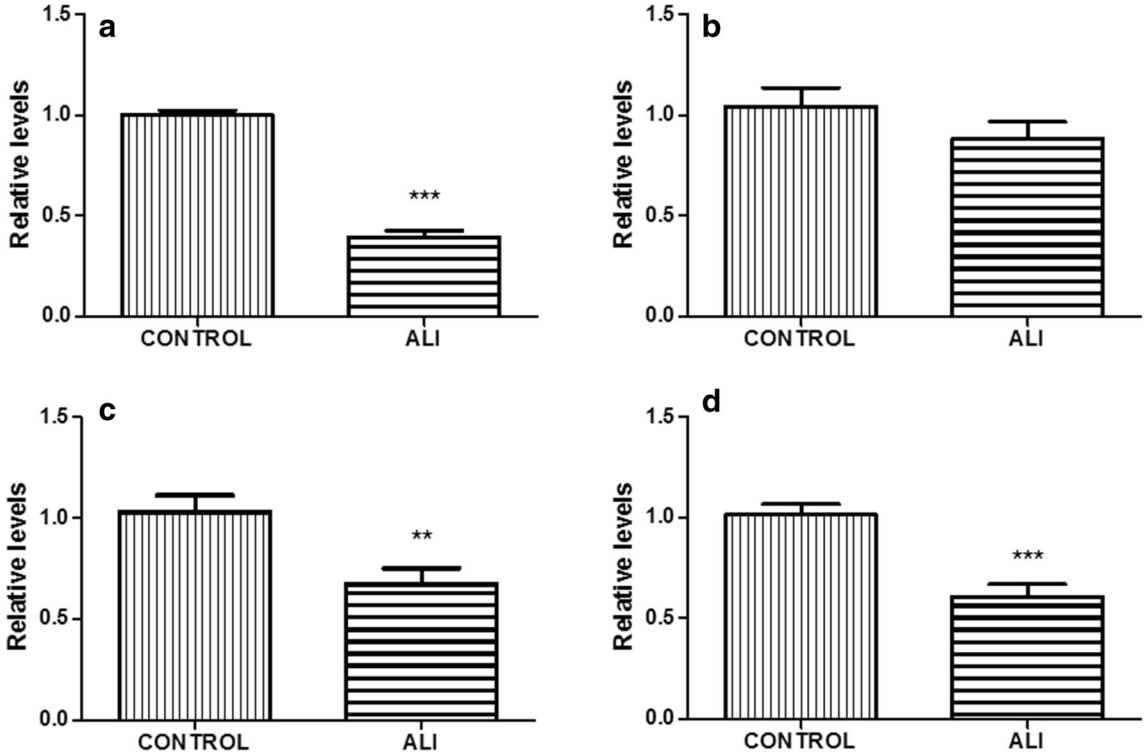

Fig. 4 mRNA relative level related to excision base repair pathway **a** OGG1 and **b** APEX1 and excision nucleotide repair pathway **c** ERCC2 and **d** ERCC1 in lung tissue after intraperitoneal induction of lipopolysaccharide (LPS). GAPDH was used as internal standard for normalization. *when compared acute lung injury (ALI) with control group. $^{**}p < 0.01$ and $^{***}p < 0.001$

exposure to infrared laser at higher fluence (20 J/cm^2) when compared to APEX1 mRNA relative levels in lung tissue from rats after sepsis-induced ALI not exposed to infrared laser.

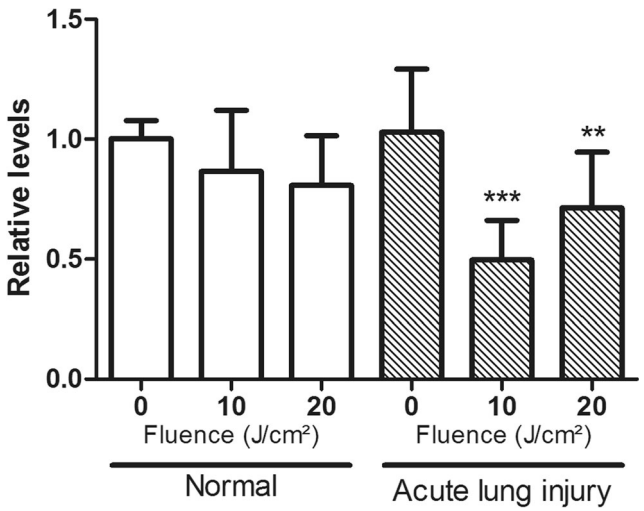

Fig. 5 OGG1 mRNA relative level in lung tissue after lipopolysaccharide (LPS) induction and low-power infrared laser exposure (808 nm). Following the groups: *Normal*: *0* (control), *10* (10 J/cm^2), *20* (20 J/cm^2); *Acute lung injury*: *0* (acute lung injury), *10* (acute lung injury and 10 J/cm^2), *20* (acute lung injury and 20 J/cm^2). GAPDH was used as internal standard for normalization and ALI group was used as the second normalizer (ΔΔCt). *when compared with ALI group. $^{**}p < 0.01$ and $^{***}p < 0.001$

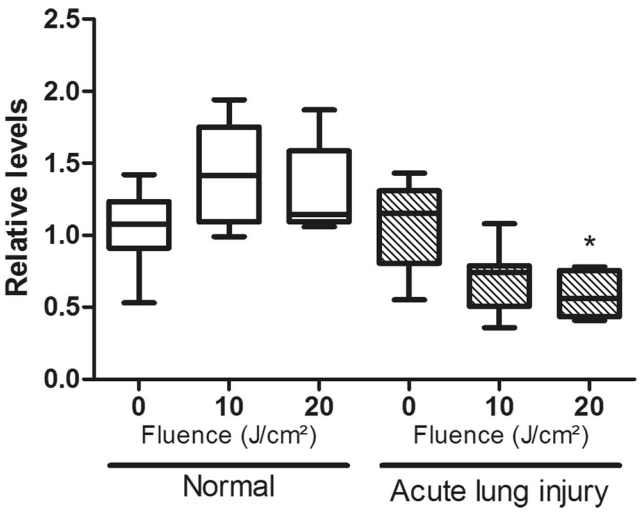

Fig. 6 APEX1 mRNA relative level in lung tissue after lipopolysaccharide (LPS) induction and low-power infrared laser exposure (808 nm). Following the groups: *Normal*: *0* (control), *10* (10 J/cm^2), *20* (20 J/cm^2); *Acute lung injury*: *0* (acute lung injury), *10* (acute lung injury and 10 J/cm^2), *20* (acute lung injury and 20 J/cm^2). GAPDH was used as internal standard for normalization and ALI group was used as the second normalizer (ΔΔCt). *when compared with ALI group. $^{*}p < 0.05$

Effects of low-power infrared laser on mRNA relative levels from nucleotide excision repair genes in lung tissue from normal and sepsis-induced ALI rats

After verifying mRNA relative levels from genes related to base excision repair pathway (OGG1 and APEX1) are altered in lung tissue after sepsis-induced ALI and infrared laser exposure, mRNA relative levels from nucleotide excision genes were evaluated.

Figure 7 shows ERCC2 mRNA relative levels in lung tissue from rats after sepsis-induced ALI and exposure to low-power infrared laser at different fluences (10 and 20 J/cm^2). Data in this figure indicate significant increase of mRNA levels in lung tissue from normal rats exposed to infrared laser at 10 and 20 J/cm^2 ($p < 0.01$ and $p < 0.05$, respectively) when compared with control group. However, no significant ($p > 0.05$) alteration of ERCC2 mRNA levels in lung tissue from rats after sepsis-induced ALI and exposed to infrared laser when compared with ERCC2 mRNA relative levels in lung tissue from rats after sepsis-induced ALI not exposed to infrared laser.

ERCC1 mRNA relative level was evaluated to confirm the alteration of mRNA relative levels from nucleotide excision repair genes (Fig. 8). Exposure to infrared laser significantly increased ($p < 0.05$) ERCC1 mRNA relative levels in lung tissue from normal rats exposed to infrared laser at the higher fluence (20 J/cm^2) when compared to control group. No significant ($p > 0.05$) differences in ERCC1 mRNA relative levels occurred in lung tissue from rats after sepsis-induced ALI and exposed to infrared laser at the lower fluence (10 J/cm^2) when compared with ERCC1 mRNA relative levels in lung tissue from rats after sepsis-induced ALI not exposed to infrared laser.

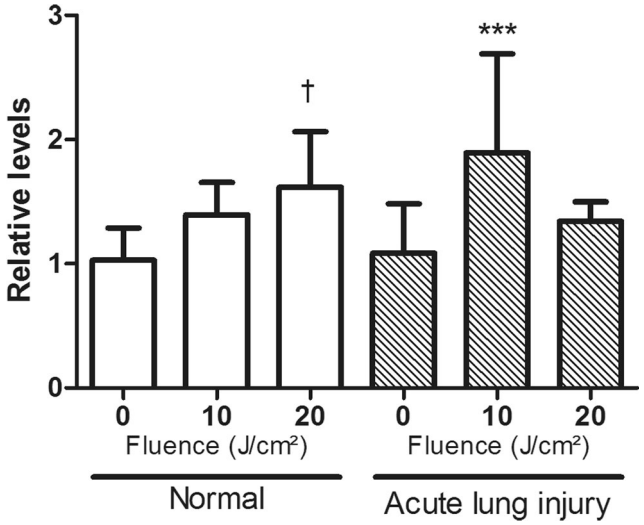

Fig. 7 ERCC2 mRNA relative level in lung tissue after lipopolysaccharide (LPS) induction and low-power infrared laser exposure (808 nm). Following the groups: *Normal*: *0* (control), *10* (10 J/cm^2), *20* (20 J/cm^2); *Acute lung injury*: *0* (acute lung injury), *10* (acute lung injury and 10 J/cm^2), *20* (acute lung injury and 20 J/cm^2). GAPDH was used as internal standard for normalization and ALI group was used as the second normalizer (ΔΔCt). †when compared with control group and *when compared with ALI group. †$p < 0.05$ and *** < 0.001

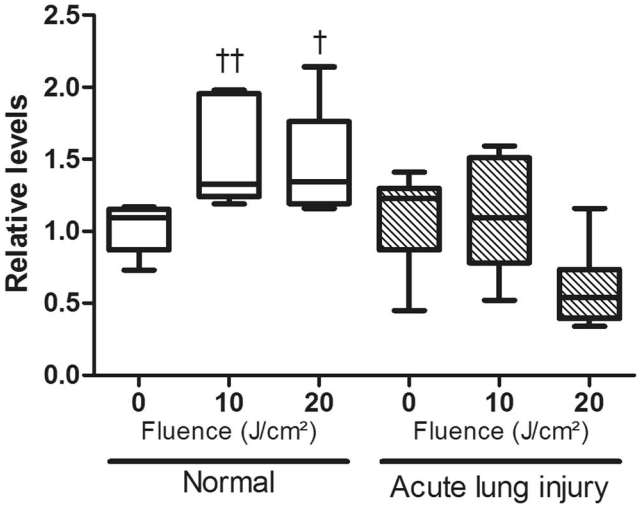

Fig. 8 ERCC1 mRNA relative level in lung tissue after lipopolysaccharide (LPS) induction and low-power infrared laser exposure (808 nm). Following the groups: *Normal*: *0* (control), *10* (10 J/cm^2), *20* (20 J/cm^2); *Acute lung injury*: *0* (acute lung injury), *10* (acute lung injury and 10 J/cm^2), *20* (acute lung injury and 20 J/cm^2). GAPDH was used as internal standard for normalization and ALI group was used as the second normalizer (ΔΔCt). †when compared with control group. †$p < 0.05$ and ††$p < 0.01$

Discussion

In ARDS/ALI by LPS administration, there is diffuse alveolar damage with decreased airways accompanied by thickening of interalveolar septa wall, with progression to fibrosis [50] as characterized in Fig. 3d. Studies with patients in sepsis conditions show a relationship between reactive oxygen species, reduction of antioxidant capacity and accumulation of oxidative stress markers [51–54] in inflammatory process established in injured lung, with activation of neutrophils causing microvascular lesions by release of proteases and production of reactive oxygen species [55–58].

Some studies suggest that the increased oxidative stress surpasses the antioxidant defenses, causing damages in cellular molecules [59, 60]. Damage in DNA molecule could be caused by increased production of reactive oxygen and nitrogen species, a consequence of sepsis in acute lung injury. Our findings show alterations in mRNA levels from genes related to base and nucleotide excision repair.

OGG1 (*8-oxoguanine DNA glycosylase*) is an enzyme with lyase activity for cleavage of the DNA strand, responsible for

the excision of 8-oxoguanine, a mutagenic byproduct that occurs as a result of exposure to reactive oxygen [61]. Our finding shows decreasing of relative mRNA levels from OGG1 in acute lung tissue from rats after sepsis-induced ALI (Fig. 4a). It has been reported that OGG1 protects against oxidant-induced mitochondrial DNA damage and apoptosis in pulmonary artery endothelial cells [62], as well protects against ventilator-induced lung injury in intact mice [63].

Different from those obtained for OGG1, there is no significant change in APEX1 mRNA levels in lung tissue from rats after sepsis-induced ALI (Fig. 4b). APEX 1 (*apurinic/apyrimidinic endodeoxyribonuclease 1)* initiates repair of apurinic/apyrimidinic sites in DNA by catalyzing hydrolytic incision of the phosphodiester backbone immediately adjacent to the lesion, generating a single-strand break with 5-deoxyribose phosphate and 3-hydroxyl ends to insertion of a new base by DNA polymerase [64].

Oxidative stress plays a key role in sepsis pathogenesis and this is associated with reduction of patient survival [65]. In fact, base excision repair is an important DNA repair pathway acting on oxidative lesions caused by free radicals attack of DNA [66]. Interestingly, relative mRNA levels from OGG1 and APEX1 genes did not increased in lung tissue affected by sepsis, despite sepsis-induced ALI increases oxidative stress in lung tissue [67]. This could be related to the fact that in lungs at sepsis conditions, there is hypoxemia with consequent reduction of oxygen and production of reactive oxygen species. Oxidative stress reported in acute lung disease is manifested in defense cells. Once the material collected was more lung cells than defense cells, this fact could explain these results.

This reduction or non-change could be associated with enzyme activity, since physiologically DNA repair capacity is related to levels of proteins involved in this process, which are controlled by gene transcription levels (mRNA levels) [68]. Therefore, basal levels of DNA repair gene transcription are related to ability of cells to repair DNA when stimulated by endogenous and exogenous agents [68, 69]. Data suggest that decreased ability to repair oxidative lesions increases the number lesions in DNA and inflammation response [70]. Reduction of enzymatic capacity could occur since polymorphisms in some non-DNA repair gene in lung tissue affected by ALI were reported [70–72]. However, polymorphisms in DNA repair genes, specifically OGG1 and APEX1 in acute lung injury conditions, are not reported yet.

ERCC2 (*excision repair cross-complementing group 2*) and ERRC1 (*excision repair cross-complementing group 1*) acts in nucleotide excision repair pathway. NER acts on non-specific oxidative lesions, whereas BER acts on specific lesions caused by DNA molecule reaction with oxidative agents, such as free radicals [73]. This protein is involved in repair of oxidative and bulky lesions, which must be removed from DNA molecule by repair mechanisms to preserve DNA coding stability [74]. ERCC1 gene product forms a heterodimer complex with XPF protein [75] and performs the 5′ incision in DNA strand at the DNA lesion site after all other excision steps have been performed [32, 76].

Figure 4c, d shows a significant reduction of ERCC2 and ERCC1 mRNA levels, respectively, in lung tissue after sepsis-induced ALI. Reduction of NER mRNA levels in lung tissue could be related to its non-specific action on oxidative DNA lesions.

Once changes in mRNA levels from base and nucleotide excision repair genes were verified in sepsis-induced ALI rats, mRNA levels from these DNA repair genes were evaluated after exposure to low-power infrared laser in lung tissue from these animals, as well in normal animals.

Despite exposure to infrared laser do not modify the mRNA levels in lung tissue from normal rats, OGG1 mRNA relative levels decrease in lung tissue from sepsis-induced ALI rats after exposure to infrared laser (Fig. 5). These results suggest that laser-induced effects on OGG1 mRNA levels depend on tissue conditions. On the other hand, OGG1 mRNA relative levels increase after exposure to infrared laser (830 nm, 10 J/cm^2) in muscle tissue from normal rats [31] and in myoblast cultures exposed to infrared laser (808 nm, 10 J/cm^2) under normal and nutritional stress condition [77].

Similarly to OGG1 mRNA levels, APEX1 mRNA relative levels is not altered in lung tissue from normal animals, but in lung tissue from sepsis-induced ALI animals, APEX1 mRNA levels is reduced after exposure to low-power infrared laser at the higher fluence used (20 J/cm^2). Studies have shown that APEX1 mRNA levels increase in normal muscle tissue after expose to infrared laser (830 nm, 1 and 5 J/cm^2), but at 10 J/cm^2 there is a reduction of APEX1 mRNA relative levels in normal skin exposed to this laser [31]. Also, infrared laser exposure (808 nm, 10, 35, and 70 J/cm^2) increases APEX1 mRNA relative levels in myoblast cultures under both normal and nutritional stress conditions [77]. Absorption of laser photons is the primary event that leads to biological effects [78], being dependent on laser wavelength and presence of chromophores in cells, such as cytochrome c oxidase, which absorbs the photons at wavelengths ranging from 600 to 1000 nm [79]. This is the so-called therapeutic window, where the efficiency of penetration and absorption of radiation in the biologic tissue is maximum because melanin absorbs photons with wavelengths below 600 nm, while water molecules absorb those with wavelengths above 1000 nm [78]. On the other hand, basic concept of biphasic dose response [80] demonstrates that lasers at low fluences or doses present photobiostimulation effect due to high production of ATP and low production of ROS contributing to transcription of genes related to therapeutic effects, such as those related to NF-kB pathway, while lasers at high fluences or doses could inhibit effects by the high production of ROS and stimulating the transcription of genes related to cell death, such as those related to apoptosis [81].

After evaluating base excision repair pathway (OGG1 and APEX1), effects of photobiomodulation on mRNA levels from nucleotide excision repair genes were evaluated to verify the action of a non-specific DNA repair pathway for oxidative lesions in lung tissue from both normal and sepsis-induced ALI rats. Different from base repair genes, ERCC2 mRNA levels increases in lung tissue from normal rats after exposure to infrared laser but not in lung tissue from sepsis-induced ALI. These results from normal rats do not agree with previous results in skin exposed to infrared laser (808 nm, 25, 50, and 100 J/cm^2), but they agree with others from muscle tissue after exposure to same laser at fluence of 50 J/cm^2 [82].

ERCC1 mRNA levels were evaluated to confirm those obtained for ERCC2 mRNA levels. In fact, there is increase in ERCC1 mRNA levels in lung tissue both normal and sepsis-induced ALI rats. Study shows that there is reduction in ERCC1 mRNA levels in skin after exposure to infrared laser (808 nm; 25, 50, and 100 J/cm^2); however, there is increase in muscle tissue when exposed to same conditions [82]. Also, in myoblast cultures under normal and nutritional stress conditions, exposure to infrared laser (808 nm; 10, 35, and 70 J/cm^2) increases ERCC1 mRNA levels and the same occurs for XPC mRNA levels at fluence 35 J/cm^2 [77].

Taken together, the similarities and discrepancies of mRNA levels from genes related DNA repair could be explained due to the different chromophores or concentration of chromophores in each tissue, as well due to conditions of these tissues. On the other hand, low-power laser beam before to reach the lungs, ran through the skin and muscle tissue, which absorb and scatter part of laser beam [83, 84]. The results obtained in this study confirm the hypothesis that low-power infrared laser modulates mRNA levels from DNA repair genes in acute lung injury.

Conclusion

Our research shows that acute lung injury induced by sepsis alter mRNA levels from genes related to base and nucleotide excision repair genes, suggesting that DNA repair is part of cell response to sepsis, and that photobiomodulation could modulate the mRNA levels from these genes in lung tissue.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Experiments were conducted in accordance with the Ethics Committee in Animal Experiments of Universidade Federal de Juiz de Fora, Minas Gerais, Brazil, protocol number 012/2016.

Informed consent Not applicable.

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