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ORIGINAL ARTICLE

Low-level laser therapy (LLLT) attenuates RhoA mRNA expression in the rat bronchi smooth muscle exposed to tumor necrosis factor-α

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Abstract Low-level laser therapy (LLLT) has been found to produce anti-inflammatory effects in a variety of disorders. Bronchial smooth muscle (BSM) hyperreactivity is associated with increased Ca^{+2} sensitivity and increased RhoA mRNA expression. In the current study, we investigated if LLLT could reduce BSM contraction force and RhoA mRNA expression in tumor necrosis factor-α (TNF-α)-induced BSM hyperreactivity. In the study, 112 male Wistar rats were divided randomly into 16 groups, and BSM was harvested and suspended in TNF-α baths for 6 and 24 h, respectively. Irradiation with LLLT was performed with a wavelength of 660 nm for 42 s with a dose of 1.3 J/cm^2. This LLLT dose was administered once in the 6-h group and twice in the 24-h group. LLLT significantly decreased contraction force in BSM at 6 h (TNF-α + LLLT: 11.65±1.10 g/100 mg of tissue) (F=3115) and at 24 h (TNF-α+LLLT: 14.15±1.1 g/100 mg of tissue) (F=3245, p<0.05) after TNF-α, respectively, when compared to vehicle-bathed groups (control). LLLT also significantly decreased the expression of RhoA mRNA in BSM segments at 6 h (1.22±0.20) (F=2820, p<0.05) and 24 h (2.13±0.20) (F=3324, p<0.05) when compared to BSM segments incubated with TNF-α without LLLT irradiation. We conclude that LLLT administered with this protocol, reduces RhoA mRNA expression and BSM contraction force in TNF-α-induced BSM hyperreactivity.

Keywords Bronchial hyperreactivity · RhoA protein · TNF · Low-level laser therapy · Rat

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Introduction

Tumor necrosis factor-α (TNF-α) is one of the pro-inflammatory cytokines produced by a variety of cells in the airways [1–4]. TNF-α is directly linked to airway inflammation and hyperreactivity of bronchial smooth muscle (BSM) beside it there are reports indicating that the pro-inflammatory of this cytokine occurs through increased calcium sensitization when the BSM are stimulated with cholinergic agonists [5, 6].

The contractions in smooth muscles may involve not only an elevation of intracellular calcium but also an enhancement of calcium sensitivity of the contractile

apparatus [7]. Is it generally accepted that the initiation of smooth muscle contractility is predominantly controlled by a Ca^{+2}-dependent increase in myosin light $chain_{20}$ (MLC_{20}) phosphorylation [8]. However, other pathways may regulate the contractility of smooth muscle by regulating the phosphorylation level of MLC_{20} independently of a rise in intracellular calcium [9]. These pathways are generally stimulated by contractile agonists that activate heterometric G protein-coupled receptors, probably via stimulation of Rho guanine exchange factors [10, 11]. Activation of the monomeric G protein RhoA by seven-transmembrane G protein-coupled receptors leads to subsequent activation of an isolated down-stream target of Rho, Rho-associated coiled-coil-containing Rho-kinase [12]. The Rho kinase directly phosphorylates the regulatory subunit of the smooth muscle myosin light chain phosphatase [13], either directly or via an additional myosin phosphatase-associated kinase [14]. This phosphorylation results in an inhibition of phosphatase activity leading to increased accumulation of phosphorylated MLC_{20} and subsequently an increased Ca^{+2} sensitivity to contraction [15].

An experimental study [16] showed that the level of RhoA, an important protein that mediates Ca^{+2} sensitization and ACh-induced Ca^{+2} sensitization in bronchial preparations from rats that had been repeatedly antigen challenged, was significantly increased when compared to those of control rats. Sakai and coworkers [17] demonstrated that TNF-α augments the expression of RhoA in the rat bronchus. Moreover, these same authors reported upregulation and translocation of RhoA mRNA expression in BSM of rats challenged with antigen [18, 19]. The relationship between Ca^{+2} sensitization and RhoA, has been demonstrated as RhoA-mediates Ca^{2+} sensitization in antigen-induced bronchial smooth-muscle hyperresponsiveness in rats [20]. These authors speculate that it is possible that the increased RhoA enhances the Ca^{+2} sensitizing signal, resulting in an augmentation of the ACh-induced contractile response in the rat airway hyperreactivity. Further testing also added support to this hypothesis as pretreatment with the Rho inhibitor C3 toxin was found to abolish Ca^{+2} sensitization [21]. Another RhoA inhibitor, Y-27632, has also been found to reverse allergen-induced AHR after the early and late asthmatic reaction [22].

Traditional asthma and lung disorder treatment protocols include corticosteroids and other disease-modifying agents such as TNF-α inhibitors [23]. Recent research findings from our group suggest that low-level laser therapy (LLLT), an alternative therapy that has been used in clinical [24] and experimental [25] studies in the treatment of inflammatory diseases, can also be effective in pulmonary inflammatory disorders in small animals [26, 27]. LLLT was shown to reduce cholinergic hyperreactivity and TNF-α mRNA expression in rat BSM segments exposure to lipopolysaccharides by an NF-kappaβ-dependent mechanism [25]. Moreover, we have recently published that LLLT attenuates the hyper-responsiveness of rat BSM segments exposure to TNF-α by reducing inositol triphosphate type-2 receptor mRNA expression impairing the accumulus of inositol triphosphate and consequently the exacerbated sensitivity to calcium [28]. Therefore, we thought that it would be relevant to test if LLLT could modulate BSM hyperreactivity through a mechanism independent from $Ca+^{2}$ release by G protein-coupled receptor activation. In this context, the present study was designed to investigate if LLLT could modulate cholinergic hyperreactivity by reducing RhoA mRNA expression in rat BSM preparations after incubation with TNF-α.

Material and methods

Animals All experiments were carried out in accordance with the guidelines of Camilo Castelo Branco University (Unicastelo) for animal care (A06/CEP/2008). The assays were accomplished on male Wistar rats weighing between 220 and 250 g each, maintained under standard conditions of temperature (22–25°C), relative humidity (40–60%), and light/dark cycle with access to food and water ad libitum. The animals were provided by the Central Animal House of the Unicastelo. All rats were placed in a common box and divided randomly into four groups of seven animals.

Treatment with tumor necrosis factor-α (TNF-α) The rats were pre-anesthetized with acepromazine (0.1 ml/kg; i.p.) and anesthetized with Zoletil (chloridrate of zolazepam, 0.1 mg/kg + tiletamine chloridrate 0.1 mg/kg i.p.). The animals were killed under deep chloral hydrate anesthesia (>400 mg/kg, i.p.) and exsanguinated by cutting the abdominal artery. The bronchi were removed via open thoracotomy, cleared of loose connective tissue, and incubated 6 or 24 h at room temperature in Dulbecco's modified Eagle's medium containing TNF-α (300 ng/ml) or Dulbecco's modified Eagle's medium alone containing without TNF-α. The medium was aerated with a continuous supplemental O_2 mixture (95% O_2/5% CO_2) during the incubation phase.

Preparation of bronchial smooth muscle (BSM) and functional study Six or 24 h after incubation with TNF-α, each bronchial segment was suspended longitudinally between stainless-steel triangular supports in 15-ml organ baths. The lower support was secured to the base of the organ and the upper support was attached via a chain to a force transducer from which isometric tension was continuously displayed on a multi-channel recorder (Ugo Basile, Italy). The bronchi were bathed in modified Krebs solution

containing (mM): 125 NaCl, 14 $NaHCO_3$, 4 KCl, 2.25 $CaCl_2$ $2H_2O$, 1.46 $MgSO_4$. $7H_2O$, 1.2 NaH_2PO_4. H_2O and 11 glucose. The baths were aerated with 5% CO_2 in oxygen, at pH 7.4 and 37°C. Passive resting tension of each BSM segment was set at 0.5 g after each tissue sample had been passively stretched to a tension of 2.0 g to optimize the resting length of each segment. During an equilibration period in the organ bath, the bronchi samples were washed three to four times at 15- to 20-min intervals. At 15 min after the last wash, the concentrations of carbachol varied from 10^{-10}M to 10^{-3}M and the dose–response curve for the BSM samples was then determined. After measurement of responsiveness to carbachol, the BSM was also depolarized with isotonic high-K^+ solution prepared by iso-osmotic replacement of NaCl by KCl in the presence of 10^{-6}M atropine and 10^{-6}M indomethacin.

Pharmacological intervention As the positive control of RhoA participation in hyperreactivity of BSM, the rats received inhalation of Y-27632 (RhoA inhibitor; 10 μM) for 3 min (Tocris Cookson Ltd., Bristol, UK); 1 h after inhalation with Y-27632, the BSM segments from rats were bathed for 6 or 24 h with TNF-α. After both periods, the BSM segments were prepared for functional studies. The procedure of inhalation used herein was equal to that described by [22].

Real time-PCR BMS was obtained from male Wistar rats and incubated with TNF-α for 6 or 24 h. PCR was performed on a 7000 Sequence Detection System (ABI Prism, Applied Biosystems, Foster City, CA) using the SYBRGreen core reaction kit (Applied Biosystems). RT-PCR was performed with specific primers for rat RhoA 195–305 (GenBank accession number X66539) forward primer 5′-CTGGTTGGGAACAAGAAGGA-3′ and reverse primer 5′-CAAAAACCTCCCTCACTCCA-3′; rat Exon (GenBank accession number D00475) and rat GAPDH-3474-3570 (GenBank accession number J00691) forward primer 5′-TTCAACGGCACAGTCAAGG-3′ and reverse primer 5′-ACATACTCAGCACCAGCATCAC-3′ as control. One microliter of RT reaction was used for real-time PCR. The PCR primer efficiencies were calculated using standard curves, and the relative expression levels of TNF-α in real time were analyzed using the 2^{CT} method, presented as the ratio to the expression of the housekeeping gene–actin.

Low-level laser therapy (LLLT) A continuous-wave diode laser (Ga-As-Al) with an output power of 2.5 mW and a wavelength of 660 nm was used. The spot size was 0.08 cm^2 and optical power density was 31.25 W/cm^2. The optical power was calibrated before and after the experiment with a Newport Multifunction Optical Meter model 1835C. After 6 h of incubation with TNF-α, the BSM segments were irradiated one unique time for 42 s at 5 min after addition of TNF-α. The LLLT dose was fixed at 1.3 J/cm^2. At the 24-h period of incubation with TNF-α, the BSM segments were irradiated in two sessions at 5 min and 6 h after incubation with TNF-α or culture medium. In each of the LLLT sessions, the BSM samples were irradiated in the wells of culture plates for 42 s with a dose of 1.3 J/cm^2.

Statistical analysis

Statistical differences were evaluated by analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test to determine differences between groups. The results were considered significant when $p<0.05$. In order to determine the analysis of variance between the experimental groups studied, two-way ANOVA statistical analysis was used to determine the F score.

Results

Effect of LLLT on BSM cholinergic hyperreactivity induced by TNF-α

Compared to vehicle-bathed control preparations, the ACh responsiveness in TNF-α (300 $ng.ml^{-1}$)-treated groups were significantly augmented in both periods of time studied. Figure 1a represents the maximal contractile response of the TNF-α-treated group at 6 h (15.33±1.61 g/100 mg of tissue), which was significantly greater than that of the maximal contractile response of vehicle-bathed group (control) (10.41±1.10 g/mg of tissue) ($F=3125$, $p<0.05$). Figure 1b shows that at 24 h after TNF-α, the maximal contractile response (18.55±1.61 g/mg of tissue) in the LLLT group was significantly greater than the contractile response of the vehicle-bathed group (control) (10.50± 1.21 g/mg of tissue) ($F=4462$, $p<0.05$). LLLT induced a significant decrease in the negative effect of TNF-α on the CCh-induced contraction in BSM at 6 h (TNF-α + LLLT: 11.65±1.10 g/100 mg of tissue) ($F=3115$) and at 24 h (TNF-α + LLLT: 14.15±1.1 g/100 mg of tissue) ($F=3245$, $p<0.05$) after TNF-α, respectively, when compared to vehicle-bathed groups (control). No other significant differences in the responses to isotonic high K^+ (10, 30, 60, and 90 mM) were observed between the groups (data not shown). Baseline BSM contractile response was measured in both saline and TNF-α (300 ng/ml) and LLLT-treated rats, but there was no significant difference in baseline BSM contractile response between the saline group and LLLT-only group. Figure 1 shows the effects after 6 or 24-h incubation with TNF-α on BSM reactivity.

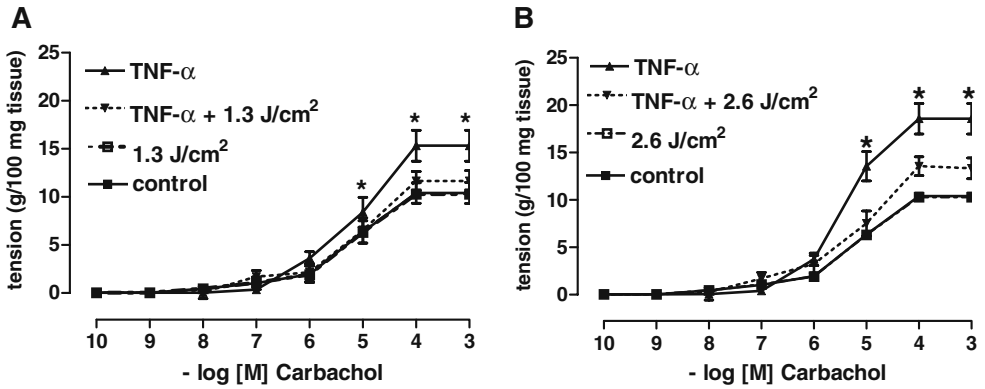

Fig. 1 Effect of PhT on cholinergic hyperreactivity of BSM induced by TNF-α. Figure 1 represents the development of tension force expressed as a dose-response curve to carbachol in different concentrations obtained before and after incubation of the bronchial muscle in TNF-α. The control group consisted of BSM segments not incubated with TNF-α and not irradiated with LLLT. The Figure 1A showed a significantly increased contractile response compared to control group 6 hours post TNF-α exposition. In the period of 6 hours bathed with TNF-α, the Figure 1A showed that LLLT radiation (1.3 J/cm^2) significantly reduced the carbachol-induced BSM tension force when compared to TNF-α group not irradiated. The Figure 1B illustrates a significantly increased contractile response compared to control group 24 hours after TNF-α. In the period of 24 hours bathed TNF-α, the Figure 1B showed that LLLT (2.6 J/cm^2) significantly reduced the carbachol-induced BSM tension force when compared to TNF-α group not irradiated. *Statistical significance, $p<0.05$ comparing TNF-α group with control; $^{\Phi}$Statistical significance, $p<0.05$ comparing TNF-α group with of TNF- α + laser

Effect of Y-27632 on cholinergic hyperreactivity of BSM induced by TNF-α

The bronchial reactivity after incubation with TNF-α at both timepoints (6 and 24 h) was significantly altered in comparison to BSM segments that were not bathed with TNF-α. At 6 h (Fig. 2a) after TNF-α incubation, the BSM hyperreactivity was augmented when compared to the control (15.40±1.63 vs. 10.41±1.10) (F=2750, $p<0.05$). The same condition of BSM hyperreactivity occurred at 24 h (Fig. 2b) after TNF-α (18.63±1.61 vs. 10.41±1.1) (F=3022, $p<0.05$). This BSM hyperreactivity was reversed 6 and 24 h after TNF-α incubation, when Y-27632 was added, and BSM reactivity returned to control values. The reactivity of BSM segments from rats treated with Y-27632, but not bathed with TNF-α, was not different from the control group. Figure 2 represents the effect of Y-27632 on BSM hyperreactivity induced 6 or 24 h after TNF-α.

Effect of LLLT on RhoA mRNA expression in BSM induced by TNF-α

At 6 h after incubation with TNF-α (300 ng.ml^{-1}), a pronounced increase occurred in RhoA mRNA expression

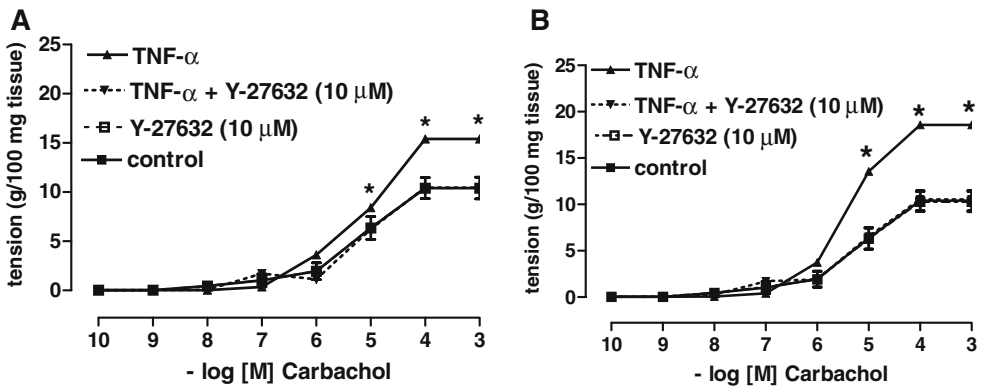

Fig. 2 Effect of Y-27632 on cholinergic hyperreactivity of BSM induced by TNF-α. The Figure 2 represents the effect of RhoA inhibitor, Y-27632, on cholinergic hyperreactivity induced by exposure of BSM rings to TNF-α during 6 (2A) or 24 hours (2B). The Figure 2A showed a significantly increased contractile response in comparison with control group 6 hours after TNF-α exposition. Six hours after TNF-α bath, the Figure 2A showed that Y-27632 significantly reduced the carbachol-induced BSM tension force to control values when compared to TNF-group. The Figure 2B showed a significantly increased contractile response compared with group control 24 hours after TNF-α exposition. In the same period, the Figure 2B showed that the Y-27632 reduced markedly the BSM reactivity to control values 24 hours after TNF-α in comparison with BSM exposure to TNF-α.. *Statistical significance, $p<0.05$ comparing TNF-α group with control; $^{\Phi}$Statistical significance, $p<0.05$ comparing TNF-α group with TNF-α + Y-27632

in BSM (3.65±0.40) in comparison to BSM not bathed with TNF-α (0.96±0.20) (F=2715, p<0.05) (Fig. 3a). At 24 h (Fig. 3b) after TNF-α exposure, RhoA mRNA expression was also augmented (5.48±0.40) in comparison to the control group (0.98±0.20) (F=3150, p<0.05), however, it was more pronounced than the 6 h when compared to each control group, respectively. LLLT significantly decreased the expression of RhoA mRNA in BSM segments at both 6 h (1.22±0.20) (F=2820, p<0.05) and 24 h (2.13±0.20) (F=3324, p<0.05) when compared to BSM segments incubated with TNF-α without LLLT irradiation. The results are summarized in Fig. 3

Discussion

This is the first report that suggests the ability of LLLT to attenuate the cholinergic bronchial hyperreactivity from Wistar rats, which may be explained through an action mechanism independent of receptor activation that reduces RhoA mRNA expression in bronchial smooth-muscle segments after exposure to TNF-α for 6 or 24 h. Recently, we reported that LLLT reduces bronchial hyperreactivity to cholinergic agonist (carbachol) by modulating Ca^{+2} sensitization and the reduction of inositol triphosphate 2 type receptor mRNA expression [28]. Pretreatment with Xestopongin C, an inhibitor of inositol triphosphate, blocked the LLLT effect. These findings suggest that the effect of LLLT on bronchial hyperreactivity is at least partially dependent of the presence of inositol triphosphate in bronchial smooth-muscle segments after exposure to TNF-α.

Our findings in the current study demonstrate that LLLT can modulate the BHR through a mechanism independent of Ca^{+2} release, possibly similar to the response of G protein-coupled receptor activation. It seems reasonable to suggest that LLLT attenuated the BSM hyperreactivity due to a reduction of protein mRNA expression, which is responsible for inhibiting MLC phosphatase. This inhibition results in the promotion of a contractile state, which is caused by Ca^{+2} sensitization of smooth-muscle contraction.

Our results are in accordance with findings from other authors [17] who have demonstrated that incubation of bronchial smooth-muscle preparation with TNF-α (300 ng/ml) significantly shifted the concentration–response curve to carbachol upwards, but not due to high K^{+} levels, as seen in the control samples.

Observations that TNF-α is released via IgE-dependent activation of mast cells or macrophages in the sensitized human lung [29] suggest that TNF-α contributes to an allergen-induced inflammatory response. One study shows that exposure of human airway smooth-muscle cells to TNF-α for 24 h potentiates the increase in cytosolic free calcium induced by contractile agonists such as carbachol and bradykinin [30]. Accordingly, TNF-α may increase bronchial smooth-muscle contractility by augmenting receptor-mediated signaling via muscarinic receptors.

The increased expression of RhoA proteins by allergic stimulation seems to enhance the Ca^{+2} sensitizing signal, resulting in an augmentation of acetylcholine-induced contractile response in antigen-challenged rat airway hyperreactivity [31]. Another study with rats showed that TNF-α incubation augments acetylcholine-induced bronchial smooth-muscle contraction [17]. Cholinergic agonists have also been reported as one of the activators of RhoA in bronchial smooth muscle [32]. TNF-α-augmented expression of the RhoA protein also occurs in rat bronchial preparations [17], and TNF-α produced by allergic stimulation appears to increase the agonist-induced Ca^{+2} sensitizing signal via an enhanced expression of the RhoA proteins.

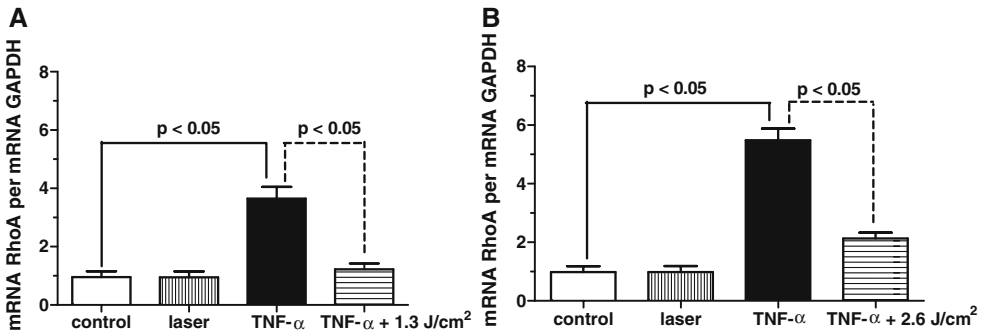

Fig. 3 Effect of LLLT on RhoA mRNA expression in BSM induced by TNF-α. Figure 3 represents the LLLT effect on RhoA mRNA expression obtained before and after incubation of the BSM with TNF-α during 6 (3A) or 24 hours (3B). The Figure 3A showed a significant increase of RhoA expression in BSM compared to control group. When BSM was treated with LLLT, the RhoA expression was deeply diminished compared to TNF-α group. The Figure 3B showed that in presence of TNF-α, the RhoA mRNA expression was increased in comparison with control group. In this same period, LLLT reduced the RhoA expression in BSM in comparison with TNF-control group (3B)

Regarding RhoA mRNA expression in bronchial smooth-muscle segments, our findings corroborate with the results obtained by [17] in which the exposure of bronchi segments to TNF-α provoked a time-dependent increase of RhoA expression, reaching a maximal value 24 h after TNF-α. These authors suggest that TNF-α might be one of the important mediators that are involved in the pathogenesis of the augmented bronchial smooth-muscle contractility in airway hyperreactivity mediated by the upregulation of RhoA in bronchial smooth muscle.

In order to investigate pharmacologically the participation of RhoA in the augmentation of bronchial contractility against cholinergic agonist, some authors have shown that a selective Rho-associated coiled coil-forming protein kinase family (ROCK) inhibitor named Y-27632 [33] decreased blood pressure of hypertensive rats [34] and inhibited smooth-muscle contraction ex vivo in rabbit aorta and in guinea pig and bovine trachea [35]. Furthermore, Y-27632 relaxes smooth muscle in the human bronchus and pulmonary artery [36]. In fact, [37] demonstrated a suppression of airway hyperresponsiveness induced by ovalbumin sensitization and respiratory syncytial virus with Y-27632. Another study demonstrated that inhaled Y-27632 protects against acute allergen-induced bronchoconstriction, development of airway hyperreactivity after the early airway reactivity and late airway reactivity, and airway inflammation in an established guinea pig model of allergic asthma [38, 39]. This suggests that RhoA is at least partially responsible for increased airway reactivity against cholinergic agonists, as is observed in asthma. In the present study, the pretreatment of bronchial smooth muscle with Y-27632 even brought the bronchial reactivity back to control values at both timepoints.

Increased responsiveness of bronchial smooth muscle has previously been demonstrated in a rat model of airway hyperreactivity induced by repeated antigen inhalation [40]. In this animal model of airway hyperreactivity, the bronchial smooth-muscle contraction induced by receptor agonists such as acetylcholine, but not K^+ depolarization, is markedly augmented [40]. Moreover, it has also been demonstrated that muscarinic receptor density and antagonist affinity of airway smooth muscle are not increased, but stay at normal levels. Thus, it is possible that the mechanism responsible for airway hyperreactivity, at least partially, can be found in the downstream pathway of muscarinic receptor signaling, including agonist-mediated Ca^{+2} sensitization. Ca^{+2} sensitization of airway smooth muscle has been reported in canine [41], porcine [42], and rabbit trachea [43], as well as human bronchus [44]. Since the Ca^{+2} sensitization induced by acetylcholine is sensitive to Y-27632, the RhoA/Rho-kinase pathway is involved in the signaling. RhoA and Rho-kinase are also expressed in rat bronchial smooth muscle [19]. Moreover, the agonist-induced increase in the cytosolic Ca^{+2} level has been reported to be more at normal levels, even in hyperreactive bronchial smooth muscles [45], reminding us that the Ca^{+2} sensitization induced by agonist stimulation might be elevated in airway hyperreactivity. Indeed, an augmentation acetylcholine-induced, RhoA-mediated Ca^{+2} sensitization of bronchial smooth-muscle contraction in a rat airway hyperreactivity model has been suggested by the following findings [31]: (1) the Ach-induced Ca^{+2} sensitizing effect, measured in permeabilized muscle strips under a constant Ca^{+2} concentration, was augmented in the airway hyperreactive rats; (2) this Ca^{+2} sensitizing effect was blocked by pretreatment with Y-27632; and (3) the RhoA protein expression in the bronchial muscle was markedly increased in the airway hyperreactivity rats. It is thus possible that RhoA/Rho-kinase-mediated signaling is the key to understanding the augmented bronchial smooth-muscle contraction in asthma.

Regarding the LLLT effects on bronchial hyperreactivity induced by TNF-α, the current study demonstrated that LLLT was efficient in reducing bronchial hyperreactivity at 6 and 24 h after TNF-α exposure. Still, LLLT was more effective in reducing the earlier bronchial hyperreactivity (6 h) than in the 24-h period of TNF-α exposure. During the 24 h-period of bronchial smooth-muscle segments incubation with TNF-α, the BSM segments were treated with LLLT at 5 min and 6 h. Although these segments received two laser irradiations, the effect was not as clearly pronounced as in the group receiving a single dose of 1.3 J/cm^2. In this condition, the exposure time of BSM segments to TNF-α may have an influence on the LLLT response, and this should be a topic for future experiments. Some reports, such as [25], described that LLLT modulates the cholinergic bronchial hyperreactivity or β_2-adrenergic hyperreactivity induced by lipopolysaccharides through an NF-κB dependent mechanism. Therefore, we cannot rule out the possibility that LLLT acts on other inflammatory mediators produced by bronchial smooth muscle after contact with TNF-α.

Considering that LLLT reduced the IP3 expression into BSM segments stimulated by TNF-α and that the IP3 generated by phospholipase C is responsible for Ca^{+2} release from the sarcoplasmic reticulum, this may suggest that the reduction of RhoA mRNA expression is an indirect effect. The precise nature of the activation of RhoA by receptor coupled to G protein is not entirely clear, but it involves guanine nucleotide exchange factors. Unfortunately, we have not yet investigated if LLLT can act directly on the myosin light chain kinase (MLCK) and MLC phosphatase [7]. The activated GTP-bound form of RhoA increases RhoA-kinase activity, subsequently leading to an inhibition of MLC phosphatase. When the MLC phosphatase is inhibited, the phosphorylated MLC cannot be dephosphory-

lated, resulting in a promotion of a contractile state of Ca^{+2} sensitization.

Conclusions

In this study, we have demonstrated that LLLT can modulate the TNF-α-induced bronchial hyperreactivity by mechanisms that may be independent of Ca^{+2} release generated by G protein-coupled receptor activation.

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