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MHTP, a synthetic tetratetrahydroisoquinoline alkaloid, attenuates lipopolysaccharide-induced acute lung injury via p38MAPK/p65NF-κB signaling pathway-TLR4 dependent

Bianka Márcia do Nascimento Xavier[1] · Larissa Adilis Maria Paiva Ferreira[1] · Laércia Karla Diega Paiva Ferreira[1] · Francisco Allysson Assis Ferreira Gadelha[1] · Talissa Mozzini Monteiro[1] · Luiz André de Araújo Silva[2] · Luís Cesar Rodrigues[2] · Marcia Regina Piuvezam[1]

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Abstract

Introduction This study investigated the mechanism of action of a synthetic tetrahydroisoquinoline alkaloid, MHTP, in an experimental model of acute lung injury (ALI) in two distinct moments: 72 h and 10 days.

Methodology To realize this study, 2.5 mg/kg of lipopolysaccharide (LPS) was intranasally administered in BALB/c mice, and nasal instillation of MHTP (1.25; 2.5; 5.0; 10 or 20 mg/kg) was administrated at 1, 24, and 48 h after LPS challenge. The data were statistically analyzed and $p < 0.05$ was considered statistically significant.

Results MHTP treatment (2.5, 5.0, 10 or 20 mg/kg) significantly decreased neutrophil migration into the bronchoalveolar lavage fluid (BALF), tissue inflammatory cell infiltration, edema, and hemorrhage as well as collagen fiber deposition on the perialveolar regions at both moments. TNF-α and IL-6 levels were significantly diminished in the MHTP-treated animals at 72 h and maintained them, at a basal level, at 10-day observation. These effects of MHTP are due to downregulating p38MAPkinese/p65NFκB signaling pathway-TLR4 dependent. Also, the MHTP treatment promoted a survival rate at 100% and improved their body weights during the 10-day observation. Unlike, the LPS group (non-treated LPS challenged animals) presented less than 50% of surviving rate at 72 h and the animals that survived did not improve their physiological state at 10-day observation.

Conclusions These data showed for the first time the beneficial and effective activity of a nasal treatment with a synthetic tetrahydroisoquinoline alkaloid on an experimental model of ALI and pointed out the molecular mechanism related to it.

Keywords MHTP · Alkaloid · Intranasal route · Toll-like receptor 4 · Cytokines · Signaling pathway

Introduction

Isoquinoline alkaloids are natural products present in several plant families and regulate biological systems [1]. For instance, typical isoquinoline alkaloids are the tetrahydroisoquinoline alkaloids commonly found in numerous structurally diverse natural products that exhibit a wide range of biological and pharmacological activities such as blocking the NFκB signaling pathway [2], antitumor effect [3] and anti-inflammatory and analgesic activities [4, 5] among others. In addition, these natural chemical structures can be synthesized and used for pharmaceutical purpose, for instance, morphine, codeine, and thebaine, which have analgesic, cough suppressing and muscle relaxant effects, respectively, as well as papaverine and noscapine that present antitumor and antimicrobial activities [6–8].

Two-methoxy-4-(7-methoxy-1,2,3,4-tetrahydrotetrahydroisoquinoline-1-yl) phenol, coded as MHTP (Fig. 1), is an unprecedented tetrahydroisoquinoline alkaloid, synthesized by our research group for prospecting new compounds with therapeutic purpose. MHTP is chemically similar to cryptostilines I, II and III isolated from *Cryptostylis fulva* (Orchidaceae) which have considerable biological effects by

✉ Marcia Regina Piuvezam
mrpiuvezam@ltf.ufpb.br

[1] Department of Physiology and Pathology, Post-Graduation Program in Natural, Synthetic and Bioactive Products, Federal University of Paraiba, João Pessoa, Paraíba, Brazil

[2] Biotechnology Center, Federal University of Paraiba, João Pessoa, Paraíba, Brazil

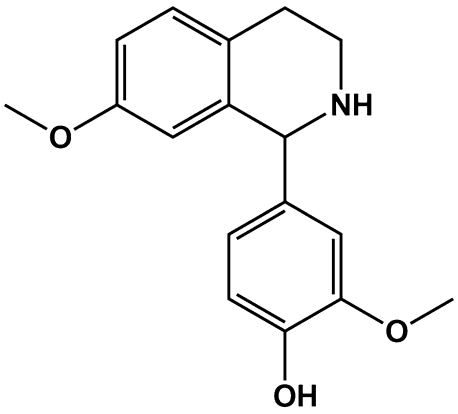


Fig. 1 The chemical structure of MHTP [2-methoxy-4-(7- methoxy-1,2,3,4-tetrahydroisoquinolin-1-yl) phenol]

being antagonists of dopamine D1 receptor [9, 10]. Also, it is chemically similar to the synthetic tetrahydroisoquinoline alkaloids THI52 (1-naphthylethyl-6,7-dihydroxy-1,2,3,4-tetrahydrotetrahydroisoquinoline) and CKD712 [(*S*)-1-(a-naphthylmethyl)-6,7-dihydroxy-1,2,3,4-tetrahydrotetrahydroisoquinoline] described as anti-inflammatory molecules [11, 12]. For instance, TH152 presents inhibitory effect on TNF-α and iNOS gene expressions observed both in vitro and in vivo experimental models [11], and CKD712 inhibits iNOS and COX-2 gene expressions on activated macrophages and presents anti-septic and anti-cancer properties [12].

Previous studies from our laboratory demonstrated that LPS-stimulated macrophages treated with MHTP diminished NO, IL-1β, IL-6 and IL-10 production, mediators involved in the inflammatory process. In in vivo inflammatory experimental models, oral administration of MHTP decreased paw edema formation by inhibiting PGE function. In addition, in carrageenan-induced peritonitis, MHTP inhibited the inflammatory cell migration, mainly neutrophils, to the peritoneal cavity as well as to the lung tissue in lipopolysaccharide (LPS)-induced acute lung injury (ALI) at a very early phase (24 h) of the illness [13]. In an experimental protocol of allergic pulmonary inflammation, nasal administration of MHTP (5 mg/kg) promoted anti-inflammatory and immunomodulatory effects by decreasing eosinophil migration to the lung tissue as well as IL-4 and IL-13 production, besides reducing TCD4+ lymphocytes in the lung cavity [14].

Acute lung injury (ALI) and its most severe form, acute respiratory distress syndrome (ARDS) are inflammatory lung conditions characterized by lung infiltration of polymorphonuclear cells (neutrophils), pulmonary endothelium and epithelial barrier disruption, loss of alveolar-capillary membrane integrity and reduction of lung compliance capacity in human [15]. The lung compliance reduction has been correlated with alveolar damage culminating with ARDS and may even lead to death [16]. The mortality rate of patients with ALI/ARDS is about 22–58% and, depending on the severity of the inflammatory process, the cause of death will be associated with multiple organ failure [17]. Currently, the ALI basis treatment is the maintenance of adequate oxygenation via mechanical ventilation, careful administration of liquids, adjunctive nutritional support and specific treatment of any organ injury [16]. However, none of these available treatments has shown a convincing improvement and, they can induce long-term side effects [17]. Therefore, it is mandatory to search for effective drugs with low side effects to control ALI/ARDS and improve the survival rate of these patients.

Natural compounds and even their synthetic prototypes are always considered candidates for the development of pharmacological drugs capable of ameliorating the various pathological condition including inflammatory processes [18, 19]. Therefore, taken all these information together, the aim of this study was to characterize mechanistically the anti-inflammatory and immunomodulatory properties, already described, of the synthetic tetrahydroisoquinoline alkaloid MHTP on acute lung injury (ALI) induced by lipopolysaccharide (LPS) in mice.

Materials and methods

Animals

Male BALB/c mice (6–8 week old) weighing between 25 and 30 g were used in the experiments. The animals, housed in polypropylene cages at a temperature of 25 ± 2 °C, were subjected to light/dark cycles of 12 h with free access to water and food during the experimental period. The animals were from Prof. Dr. Thomas George Animal Supplies of Institute of Drugs and Medicines from the Federal University of Paraíba, João Pessoa, PB, Brazil. All experimental procedures were conducted in accordance with the guidelines of the National Animal Experimentation Control Council (CONCEA) and adhered strictly to the guidelines of the Brazilian Law no. 11.794/2008, which establishes rules for use and care of laboratory animals. All experimental protocols were approved by the Ethical Committee from the Center of Health Sciences from the Federal University of Paraiba, Brazil (protocol no. 014/2017). Each experimental group consisted of five to eight mice depending on the experimental protocol.

Murine model of acute lung injury (ALI) induced by lipopolysaccharide (LPS)

Acute lung injury in mice was established as previously reported [13, 20]. Briefly, anesthetized mice were

previously challenged, intranasally, with 40 µL of LPS (2.5 mg/kg LPS—*Escherichia coli* 0111: B4-Sigma-Aldrich®) diluted in sterile saline solution (LPS group – LPS-challenged mice). The basal group received 40 µL of the sterile saline. For the MHTP groups, the LPS-challenged mice received, by intranasal route, 40 µL of different doses of MHTP (1.25, 2.5, 5, 10 or 20 mg/kg) 1 h, 24 h and 48 h after LPS challenge. The animals were maintained under normal feeding and water conditions during the experiment period and, according to the guidelines of the National Animal Experimentation Control Council (CONCEA), the animals were euthanized with an overdose of ketamine/xylazine anesthetic solution to minimize pain, stress, and suffering of the animals.

Preparation of biological material for analyses

The analysis of the inflammatory parameters was realized in two distinct moments: 72 h and 10 days after LPS challenge. For the bronchoalveolar lavage fluid (BALF) collection, the animals were euthanized with an anesthetic overdose and a longitudinal incision, in the cervical-ventral region was made to expose the animal's trachea. For better visualization of the trachea, the lobes of the thyroid gland were separated and a peripheral IV-18G polyurethane catheter was inserted into the trachea, which was connected to a 1.0 mL syringe containing ice-cold HBSS−/− and the BALF was collected and maintained in a cold-water bath. For lung collection, mice were euthanized, the thoracic cavity of the animal was opened at the border of the ribs with the diaphragm, and cardiac perfusion was performed. The perfusion consisted of the administration of 10 mL of saline solution to the heart to remove blood from the bloodstream and lavage of the lung. Both lung lobes, right and left, were collected.

Total and differential cell count

An aliquot of BALF was diluted in Turk solution and the cell suspension was counted in the hemocytometric chamber (Neubauer) using the optical microscope (40×-BX40, OLYMPUS). In addition, the BALF was centrifuged at 1500 rpm at 4 °C for 5 min., the supernatant was removed and stored for cytokine quantification. The BALF pellet was resuspended in 500 µL of ice-cold HBSS and, an aliquot of the cell suspension was added into an appropriate slide by cytospin centrifugation. The cells were fixed and then were stained with the panoptic kit. The leukocytes were differentiated into lymphocytes, macrophages, and neutrophils. Each slide was carefully analyzed by 100× objective using immersion oil and an optical microscope (100×-BX40, OLYMPUS).

Measurement of cytokine levels

Cytokine quantification (TNF-α and IL-6) from the BALF was determined by mouse-specific sandwich ELISA, according to the manufacturer's instructions (eBioscience). Optical density was read using a microplate spectrophotometer at 450 nm (microplate reader VersaMax, tunable, BN 2529, Molecular Devices).

Lung histological procedure

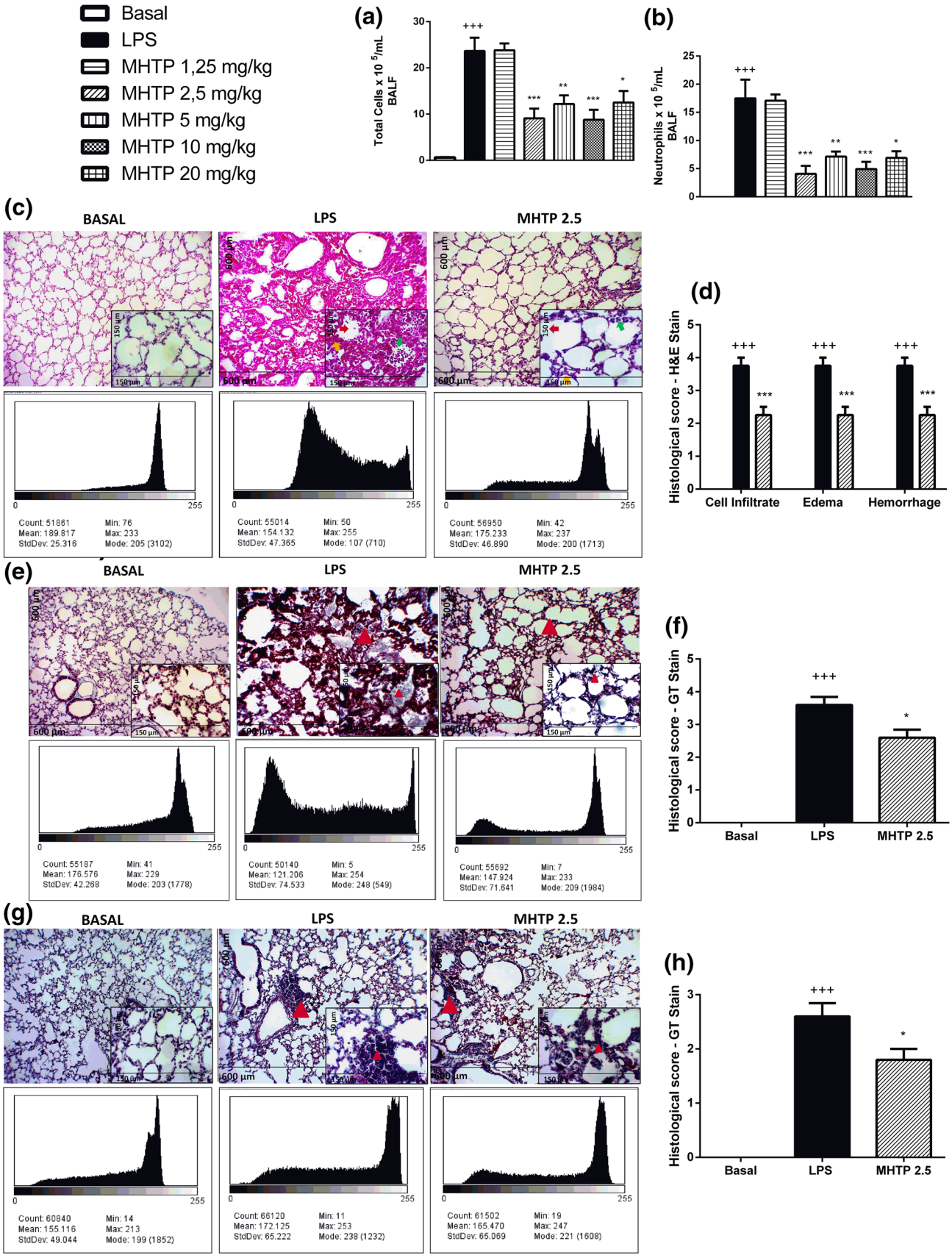
Immediately after the lung collection, both lung lobes, right and left, were submitted to formalin fixation buffered, dehydrated in ethyl alcohol, diaphanization in xylol and impregnated into paraffin. Histological sections of the lung were made on the microtome with a thickness of 5 µm. The slides with the lung histological sections were placed in a heating plate (60 °C) for 10 min and the tissue stained with hematoxylin and eosin (H&E) stain or Gömöri trichrome (GT) stain. Histological parameters as cell infiltration, edema, and hemorrhage and collagen fiber deposition were described by assigning scores according of the color intensity. The histological scores are: 0—absence of histological changes; 1—mild alterations: less than 25% of the microscopic field; 2—moderate alterations: 25–49% of the microscopic field; 3—marked degree alterations: 50–75% of the microscopic field; 4—very marked degree alterations: over 75% of the microscopic field). Representative photomicrographs of the slides and pixels were analyzed using a camera-coupled microscope and ImageJ plus 2.0 software.

Survival and body weight analysis

MHTP-treated or non-treated LPS-challenged mice were observed for death and body weighed gain during 10 days. The protocol allowed the assessment of body weight change and the survival rate of each animal group.

Cell signaling pathway analysis

Inflammatory cells from the bronchoalveolar lavage fluid (BALF) (1×10^6/mL) were incubated with anti-mouse TLR4 PE conjugated, p38MAPK PE-Cy7 conjugated or p65NFκB PE conjugated, all of them from R&D Systems (San Diego, California, USA) for 30 min at 4 °C. Cells were washed twice with cold PBS and they were resuspended in PBS. Ten thousand events were analyzed using a Becton–Dickinson FACS Canto II. The data were analyzed using Flow Jo software. Cell populations were identified according to criteria described by the manufacture. MHTP-treated or non-treated LPS-challenged mice were observed for death and


Basal
LPS
MHTP 1,25 mg/kg
MHTP 2,5 mg/kg
MHTP 5 mg/kg
MHTP 10 mg/kg
MHTP 20 mg/kg
(a)
Total Cells x 10 5/mL BALF
(b)
Neutrophils x 10 5/mL BALF
(c)
BASAL
LPS
MHTP 2.5
Count: 51861 Mean: 189.817 StdDev: 25.316 Min: 76 Max: 233 Mode: 205 (3102)
Count: 55014 Mean: 154.132 StdDev: 47.365 Min: 50 Max: 255 Mode: 107 (710)
Count: 56950 Mean: 175.233 StdDev: 46.890 Min: 42 Max: 237 Mode: 200 (1713)
(d)
Histological score - H&E Stain
Cell Infiltrate
Edema
Hemorrhage
(e)
BASAL
LPS
MHTP 2.5
Count: 55187 Mean: 176.576 StdDev: 42.268 Min: 41 Max: 229 Mode: 203 (1778)
Count: 50140 Mean: 121.206 StdDev: 74.533 Min: 5 Max: 254 Mode: 248 (549)
Count: 55692 Mean: 147.924 StdDev: 71.641 Min: 7 Max: 233 Mode: 209 (1984)
(f)
Histological score - GT Stain
Basal
LPS
MHTP 2.5
(g)
BASAL
LPS
MHTP 2.5
Count: 60840 Mean: 155.116 StdDev: 49.044 Min: 14 Max: 213 Mode: 199 (1852)
Count: 66120 Mean: 172.125 StdDev: 65.222 Min: 11 Max: 253 Mode: 238 (1232)
Count: 61502 Mean: 165.470 StdDev: 65.069 Min: 19 Max: 247 Mode: 221 (1608)
(h)
Histological score - GT Stain
Basal
LPS
MHTP 2.5

◂**Fig. 2** Effect of MHTP treatment on inflammatory cell migration into the bronchoalveolar lavage fluid (BALF) and lung tissue. BALB/c mice ($n=6$) were challenged with LPS and 1, 24 and 48 h they were nasally treated with MHTP (1.25, 2.5, 5, 10 or 20 mg/kg). BALF and lung tissues were collected 72 h or 10 days after LPS-challenge. **a** Total cells; **b** neutrophils; **c** lung tissue stained with hematoxylin and eosin (H&E): inflammatory cells (green arrow), edema (red arrow) and hemorrhage (yellow arrow); **d** inflammatory score; **e** lung tissue stained with Gomori Tricrome (GT) at 72 h: collagen fiber (red triangle); **f** collagen fiber score; **g** lung tissue stained with Gomori Tricrome (GT) at 10 days: collagen fiber (red triangle); **h** collagen fiber score. The data were expressed as mean ± SEM. The difference between the groups was analyzed by one-way ANOVA, followed by the Tukey variance test and Kruskal–Wallis. $^{+++}p<0.001$, LPS group compared to the Basal group; $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$, MHTP groups compared to the LPS group (color figure online)

body weighed gain during 10 days. The protocol allowed the assessment of body weight change and the survival rate of each animal group.

Statistical analysis

Results were expressed as mean ± S.P.M. (mean standard error). The GraphPad Prism version 5.0 software was used and values with $p<0.05$ were considered significant (GraphPad Software Inc., San Diego, USA). The one-way ANOVA variance test was used with Tukey's multiple variance post-test, Kruskal–Wallis (histological scores). Survival data were analyzed by the Kaplan–Meier method and comparisons were made using the log-rank test. The pixel analyses were realized from the photomicrographs by ImageJ software.

Results

Effect of MHTP treatment on inflammatory cell migration and collagen fiber deposition into the lung of LPS-challenged mice

Acute lung injury (ALI) is defined by migration of inflammatory cells mainly polymorphonuclear cells (neutrophils) into the bronchoalveolar cavity and lung tissue of LPS-challenged mice. Mice exposed to LPS (LPS group) showed significant ($p<0.001$) increase of total inflammatory cells dependent of neutrophils on the bronchoalveolar lavage fluid (BALF) at 72 h of LPS exposure and the treatment of these animals with MHTP (2.5, 5, 10 or 20 mg/kg, MHTP groups) decreased significantly ($p<0.05$–0.001) the number of these cell population (Fig. 2a, b). In addition, there was non-statistical difference among the effective doses; therefore, MHTP at dose of 2.5 mg/kg was chosen to further analysis. The histological analyses demonstrated that lung tissues from animals of MHTP group stained with H&E showed significant ($p<0.001$) inhibition of inflammatory cell infiltration, edema and hemorrhage as compared with animals from the LPS group (Fig. 2c, d). Another important lung tissue alteration on the experimental model of ALI is the collagen fiber deposition on the connective tissue of alveoli (perialveolar region) right after LPS challenge. Thus, the Gömöri trichrome stain (GT) of lung tissues from animals of MHTP group demonstrated significant ($p<0.05$) decrease of collagen fiber deposition on perialveolar region as compared to LPS group (Fig. 2e, f) at 72 h as well as at 10-day observation (Fig. 2g, h).

Effect of MHTP treatment on cytokine production of LPS-challenged mice

Inflammatory cytokines as TNF-β and IL-6 are present in the bronchoalveolar lavage (BALF) of LPS-challenged mice and are biomarker for this illness (Fig. 3). The MHTP group presented significant decrease of TNF-α ($p<0.0001$) and IL-6 ($p<0.05$) production as compared with LPS-challenged animals (LPS group) at 72 h and maintained these cytokines at a basal line at 10-day observation (Fig. 3a, b, respectively).

Effect of MHTP treatment on the survival rate and on the body weight of LPS-challenged mice

The survival rate of mice from all groups was evaluated and it was observed that more than 50% of animals from the LPS group died at 72 h observation compared with non-LPS-challenged mice (basal group). On the other hand, the MHTP group presented a survival rate of 100% at the same period (Fig. 4a). In addition, the animals from the MHTP group recovered their body weights during the 10-day observation unlike animals that survived from the LPS group (Fig. 4b).

Effect of MHTP treatment on TLR4 (Toll-like receptor 4)/MAPKinase/NFκB axis

The production of several inflammatory mediators as cytokines in LPS-induced ALI is, in general, dependent on Toll-like receptor 4 (TLR4)/MAPKinase/NFκB axis. Therefore, we analyzed BALF cells from both groups LPS and MHTP by looking at TLR4 cell surface, p38MAPKinase and p65NFκB expression. As we can observe in Fig. 5, the MHTP group presented significant ($p<0.05$) decrease of p38MAPK and p65NFκB expression dependently of diminishing TLR4 cell surface expression as compared with BALF cells from LPS group. These data indicated the MHTP effect is mainly by downregulating p38MAPKinase/p65NFκB signaling pathway-TLR4 dependent.

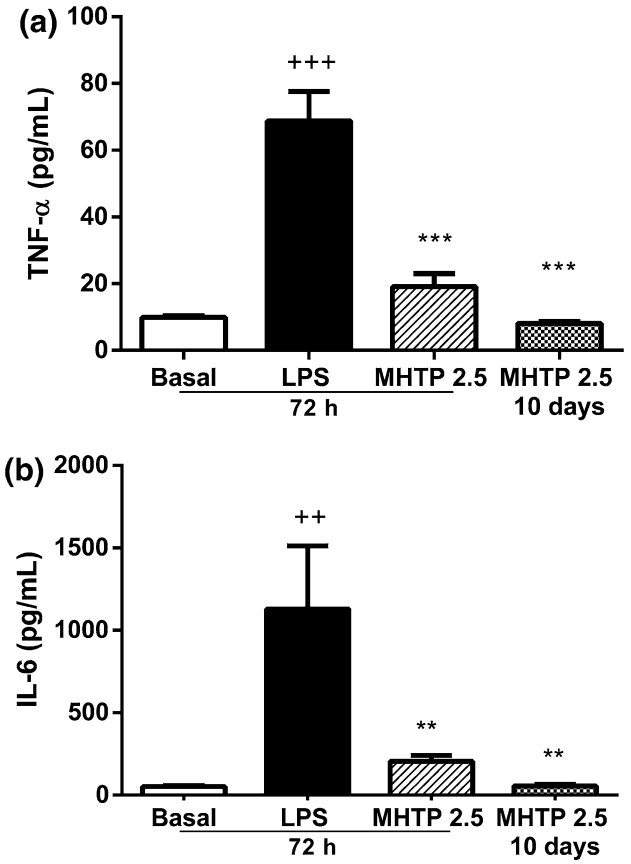


Fig. 3 Effect of MHTP treatment on inflammatory cytokine production on LPS-challenged mice. BALB/c mice ($n=6$) were challenged with LPS and 1, 24 and 48 h they were nasally treated with MHTP (2.5 mg/kg). BALF was collected 72 h and 10 days after LPS-challenge and cytokines were measured by ELISE assay (pg/mL). **a** TNF-α; **b** IL-6. The data were expressed as mean ± SEM. The difference between the groups was analyzed by one-way ANOVA, followed by the Tukey variance test. $^{+++}p<0.001$, $^{++}p<0.01$, LPS group compared to the basal group. $^{*}p<0.05$ and $^{***}p<0.001$, MHTP group compared to the LPS group

Discussion

Acute lung injury (ALI) is an acute respiratory failure-related disease that results from the destruction of the alveolar-capillary barrier associated with multiple clinical disorders, high mortality rate without specific and effective treatment [21]. Therefore, therapeutic strategies are urgently required to treat this disease.

In this perspective, MHTP, a synthetic tetrahydroisoquinoline alkaloid, was investigated in an acute lung injury experimental model (lipopolysaccharide-induced ALI) because in a preliminary study, we demonstrated a beneficial effect of this molecule on the very early phase (24 h) of the disease, by diminishing neutrophil migration and inflammatory cytokine production into the lung cavity [13]. Thus, to better understand this effect, in this study, we used the same experimental model to demonstrate the mechanism of action of the MHTP activity and may introduce it as a pharmacological prototype with a known specific pharmacological target to treat this illness.

The physiopathology of ALI is described as an increase of lung microvascular permeability, alveolar barrier dysfunction, neutrophil migration, edema, exudation, hemorrhage and collagen fiber deposition on the perialveolar regions as well as pulmonary endothelium and epithelial barrier disruption with reduction of lung compliance [15–17]. The analyses of these biomarkers of ALI makes possible to study the pharmacological potential of natural as well as synthetic molecules by defining their targets on the inflammatory process [21, 22].

Therefore, in this study, we used the nasal route to treat the LPS-challenged animals with MHTP and used some ALI biomarker analysis in two distinct moments: 72 h and 10 days. Thus, MHTP-treated animals presented a decrease of neutrophil migration, edema and hemorrhage formation at the lung tissue as well as diminished the collagen fiber deposition on the perialveolar space at 72 h and 10-day observation. The inhibition of these biomarkers by MHTP on ALI, indicates downregulation of the lung immune response. Indeed, there are pieces of evidence suggesting a critical role of activated neutrophils in the pathogenesis of most cases of ALI where these cells can release several potentially injurious metabolites, including proteases, reactive oxygen and nitrogen species, cytokines, and growth factors [23, 24]. Hence, decreasing neutrophil migration into the perialveolar space seems to be crucial to the resolution of the disease.

In addition, neutrophils and alveolar macrophages in ALI are responsible for the production of several inflammatory mediators as cytokines, IL-6 and TNF-α [24]. Several studies have shown that these two cytokines participate in the early development of ALI [25, 26] and high level of circulating IL-6 has been associated with mortality in patients with ARDS, sepsis, and trauma [27]. Besides, TNF-α is described as the first produced pro-inflammatory endogenous mediators in the ALI [25]. When TNF-α binds to its receptor on lung tissue leads to the release of enzymes causing damage to the lung parenchyma [25] as well as induces the expression of adhesion molecules in vascular endothelial cells resulting in the recruitment of neutrophils to the inflammatory site [28]. Thus, inhibition of TNF-α production is a key mechanism to the ALI control. Indeed, these authors demonstrated that administration of anti-TNF-α in LPS-challenged mice in ALI experimental model reduced the lung injury. Therefore, in this study, we observed that MHTP treatment decreased both cytokine production at 72 h and maintained them in a basal line until 10-day observation after LPS challenge. Therefore, inhibition of these inflammatory cytokines by nasal instillation of MHTP on LPS-challenged mice indicates that one of the mechanisms of action of this synthetic

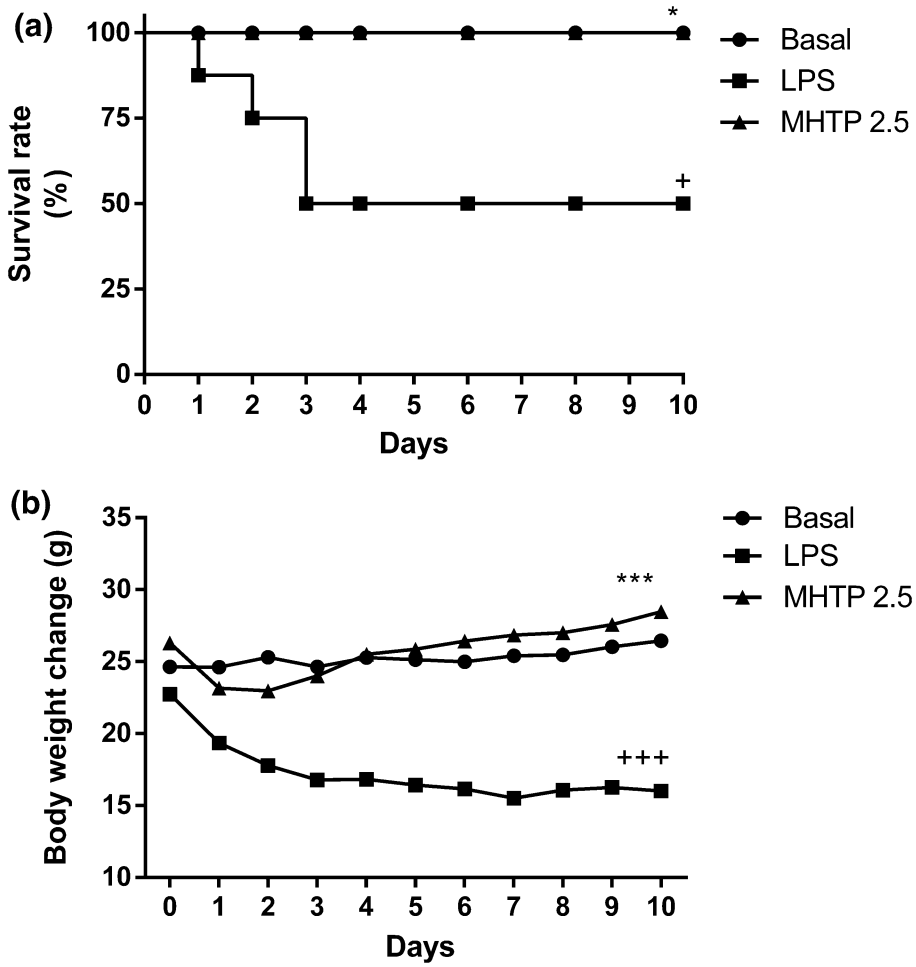


Fig. 4 Effect of MHTP treatment on the survival rate and on the body weight of LPS-challenged mice. BALB/c mice ($n=8$) were challenged with LPS and 1, 24 and 48 they were nasally treated with MHTP (2.5 mg/kg). **a** Survival rate (%); **b** body weight (g) of each animal from each experimental group (basal, LPS and MHTP) were measure during 10 days. The difference among the groups was analyzed by Kaplan–Meier method and comparisons were made using the log-rank test. $^{+}p<0.05$, $^{+++}p<0.001$, LPS group compared to the Basal group; $^{*}p<0.05$ and $^{***}p<0.001$ MHTP group compared to the LPS group

alkaloid is by inhibiting the signaling pathways related to their production.

Considering that, the production of pro-inflammatory cytokines is related to MAPKinases and nuclear transcription factors; we analyzed two signaling pathway molecule expression (p38MAPKinase and p65NFκB) as well as the TLR4 cell surface expression.

The LPS is the inductor of acute lung injury by intracellular phosphorylation processes in several lung resident cells [29]. Among the phosphorylation axis are p38MAPK/p65NFκB activation-MyD88-dependent or -independent pathways that induce the expression of several pro-inflammatory cytokine gens [30]. Therefore, we analyzed the MHTP effect on p38MAPK/p65NFκB signaling pathway-TLR4-dependent expression on cells from BALF of both animal groups (LPS and MHTP groups). Surprisingly, the MHTP treatment decreased the TLR4 cell surface expression as well as p38MAPK/p65NFκB, indicating that the effect of MHTP on ALI is, in part, by downregulating the TLR4 cell surface expression and consequently interfering with the p38MAPK/p65NFκB signaling pathway axis. Indeed, inhibition of MyD88/TIRAP/IRAK4 signaling pathway-TLR4 dependent inhibits p38MAPK activation that abrogates completely endotoxin-induced inflammation [30, 31].

Another aspect of the experimental model of ALI is the resolving phase that usually happens on 10 days [32]. To analyze the effect of MHTP in this period, the body weight rate of the animals was measured. Indeed, 100% of the animals from the MHTP group survived and improved their body weights as well as lung tissue histological aspect. In contrast, the LPS group presented a survival rate less than 50% and, the survived animals showed piloerection, difficulty to breath (data not shown) and did not improved their body weights. These data indicate clearly that MHTP protected the animals from the death and improved their life condition. Also, in the resolving phase (10 days) of ALI, the collagen fiber deposition may have decreased in the perialveolar space [33]. Indeed, nasal treatment with MHTP decreased the collagen fiber deposition on the perialveolar space of LPS-challenged mice suggesting that part of the resolution process induced by the alkaloid is due to inhibition of collagen production; therefore, further study should be done to confirm this hypothesis.

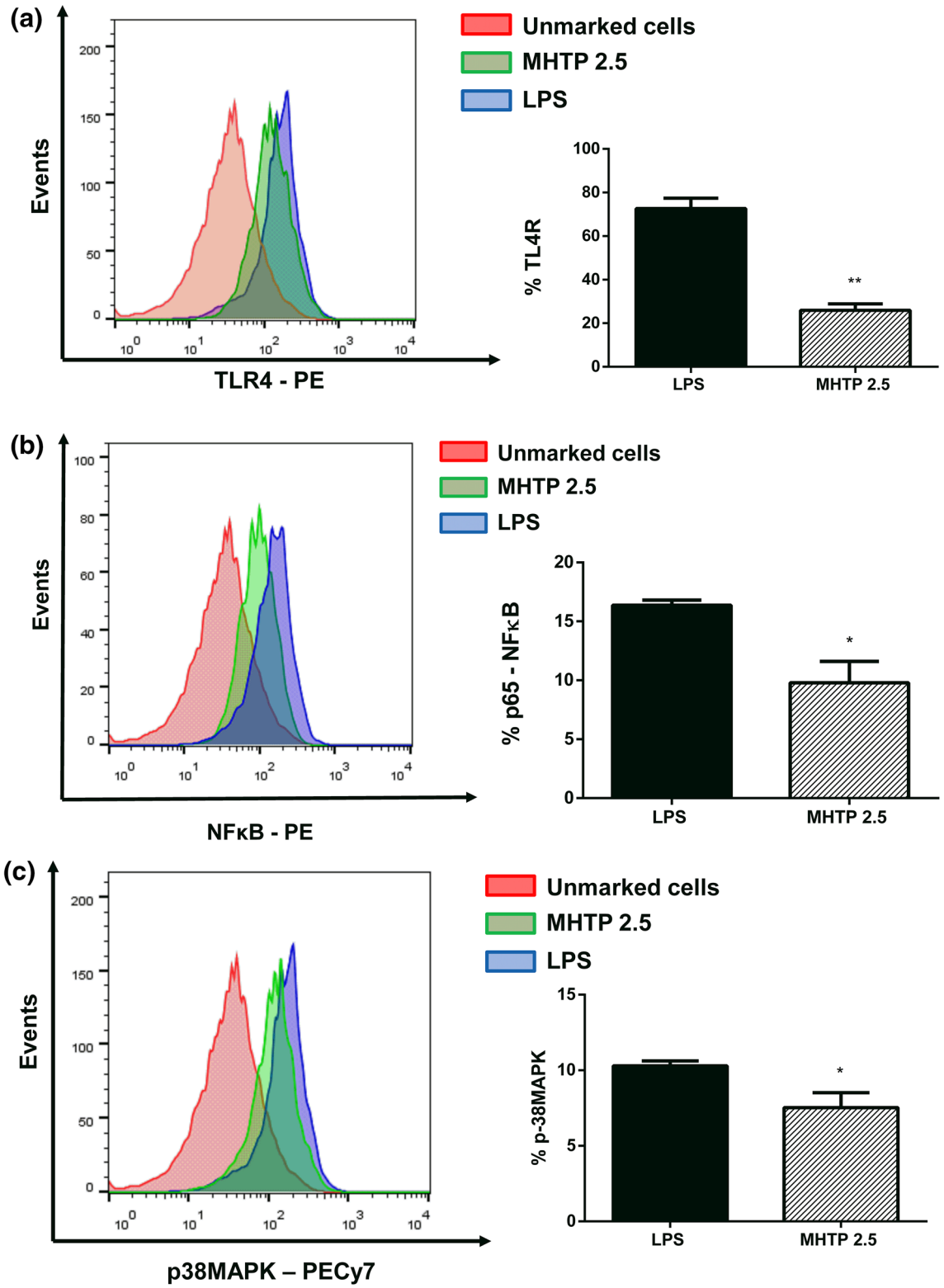


Fig. 5 Effect of MHTP treatment on toll-like receptor (TLR4) cell surface, p65NFκB and p38MAPkinase expression on LPS-challenged mice. BALB/c mice ($n=6$) were challenged with LPS (LPS group) and 1, 24 and 48 h they were nasally treated with MHTP (2.5 mg/kg). Inflammatory cells from the BALF were collected 72 h after LPS-challenged and **a** TLR4, **b** p65NFκB and **c** p38MAPk were analyzed by flow cytometry (representative histograms). The data were expressed as mean ± SEM. The difference between the groups was analyzed by one-way ANOVA, followed by the Tukey variance test. *$p<0.05$ and **$p<0.001$, MHTP group compared to the LPS group

According to the results described above, the synthetic tetrahydroisoquinoline alkaloid MHTP presented immunomodulatory and anti-inflammatory effects in the experimental model of ALI by decreasing neutrophil migration, edema, and hemorrhage and collagen fiber deposition into the perialveolar space as well as TNF-α and IL-6 production. These effects are related to downregulation of p38MAPkines/p65NFκB signaling pathway-TLR4 dependent. In addition, the nasal treatment with MHTP improved, in a long-term, the body weights and lung histological aspects of the animals as well as their life condition.

Thus, the MHTP is the first synthetic tetrahydroisoquinoline alkaloid been tested on ALI and proved to be a strong pharmacological prototype to be tested in clinical trials.

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Author contributions MRP, BMNX and LAMPF conceived and designed the experiments. BMNX, LAMPF, and LKDPF performed experimental model of acute lung injury. BMNX, LAMPF, LKDPF, and TMM performed ELISA and flow cytometry experiments. LAAS and LCR performed the synthesis of MHTP. BMNX, LAMPF, and FAAFG performed the lung histology. BMNX, LAMPF, LKDPF, and MRP analyzed the data and MRP wrote the paper.

Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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