Original Article

J Babol Univ Med Sci
23; 2021. P: 229-235

Antioxidant Effect of Montelukast on Acute Lung Injury Induced by Lipopolysaccharide in Dogs

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J Babol Univ Med Sci; 23; 2021; PP: 229-235

Received: Sep 16th 2020, Revised: Nov 7th 2020, Accepted: Dec 26th 2020.

ABSTRACT

BACKGROUND AND OBJECTIVE: Acute lung injury is characterized by accumulation of neutrophils in the lung, interstitial edema, and damage to the alveolar epithelium. Lipopolysaccharide (LPS) causes an inflammatory response and the release of reactive oxygen species and cellular and tissue damage to the lungs. Considering the role of oxidative stress in infections and proving the antioxidant properties of montelukast in several studies, the effect of montelukast on acute lung injury induced by lipopolysaccharide (as a model of infection) in dogs was investigated in this study.

METHODS: In this experimental study, 20 healthy dogs (both male and female dogs of native breed with an average weight of 20 kg) were divided into four equal groups. The first group received oral montelukast (1 mg/kg), the second group received intravenous LPS (0.1 µg/kg), the third group received montelukast one hour before LPS and the fourth group received montelukast one hour after LPS. Bronchoalveolar lavage and blood sampling were performed at hour zero and 1.5 hours after the start of the test and the amount of malondialdehyde, catalase activity, glutathione peroxidase and total antioxidant capacity in serum and lavage fluid were measured using a kit.

FINDINGS: LPS significantly increased malondialdehyde levels (from 10.5 to 139.8 µmol) and decreased catalase activity (from 0.018 to 0.007 µmol) ($p= 0.0001$), glutathione peroxidase (from 259 to 76.5 nmol) and the total antioxidant capacity (from 0.41 to 0.04 nmol) compared to hour zero. These changes were significantly adjusted by montelukast ($p\leq 0.02$).

CONCLUSION: The results of this study showed that montelukast can enhance antioxidant defense against acute lung injury induced by LPS.

KEY WORDS: *Acute Lung Injury, Oxidative Stress, Dogs, Lipopolysaccharide, Montelukast.*

Please cite this article as follows:

Soltanieh A, Avizeh R, Najafzadeh Varzi H, Razi Jalali M, Ghorbanpour M. Antioxidant Effect of Montelukast on Acute Lung Injury Induced by Lipopolysaccharide in Dogs. J Babol Univ Med Sci. 2021; 23: 229-235.

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[DOR: 20.1001.1.15614107.1400.23.1.33.2]

[DOI: 10.22088/jbums.23.1.229]

Introduction

Acute lung injury (ALI) is a critical syndrome characterized by accumulation of neutrophils in the lung, interstitial edema, and damage to the alveolar epithelium. LPS has the ability to release inflammatory cytokines and cause lung damage, which is a well-known experimental model for evaluating the effects of drugs on ALI. During ALI, the number of leukocytes, mainly neutrophils and macrophages, increases significantly in the bronchoalveolar lavage fluid in the experimental model of ALI and in patients with acute respiratory distress syndrome (1).

Free radicals are the cause of numerous organ injury and dysfunction during sepsis and are involved in pathogenesis. Lipid peroxidation mediated by oxygen free radicals is an important factor in cell membrane damage (2). LPS activates inflammatory cells and subsequently enhances the inflammatory response by releasing various cytokines such as tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1) (3). This systemic inflammatory cascade causes vascular dysfunction as well as parenchymal cell dysfunction (4).

Oxidative stress is a state of imbalance between free radicals and the antioxidant defense system (SOD, CAT and GSH surface activity) that leads to lipid peroxidation and inactivation of several enzymes (5). The antioxidant system protects cells from damage caused by free radicals such as superoxide anion and hydrogen peroxide (6). Montelukast is a selective leukotriene receptor antagonist that specifically inhibits the cysteine Leukotriene receptor (CysLT1) and reduces airway eosinophilic inflammation (7). Anti-leukotriene and leukotriene receptor antagonists are effective in several inflammatory models in mice, such as ethanol-induced gastric mucosal injury and sepsis-induced multi-organ dysfunction and ischemia/reperfusion injury (IRI) (8).

Şener et al. reported that montelukast has an anti-inflammatory effect on sepsis-induced hepatic and intestinal damage and protects against oxidative damage by a neutrophil-dependent mechanism (9). The researchers also found that montelukast has significant antioxidant effects to prevent lipid peroxidation in some tissues such as testes, liver and kidneys (10-12). Chen et al. showed that montelukast reduces the production of cytokines TNFα, IL-6 and IL-1β and increases the activity of superoxide dismutase enzyme and also reduces the amount of malondialdehyde as an indicator of oxidative stress (13). Visitsunthorn et al. concluded in their study that montelukast has a therapeutic effect in children with asthma (14). LPS increases the spread of neutrophils and increases the amount of malondialdehyde in the lung tissue (8). The aim of this study was to evaluate the anti-inflammatory and antioxidant effects of montelukast in reducing the complications of acute lung injury induced by lipopolysaccharide. The antioxidant effects were discussed in this article. In addition, due to the repetition of lavage fluid collection and the volume of the required biological samples in the present experimental study, dog was used as an animal model.

Methods

After approval by the ethics committee of Shahid Chamran University of Ahvaz with ethics code EE/99.3.02.47195/scu.ac.ir, this experimental study based on animal model was conducted on twenty adult and clinically healthy dogs aged 12-24 months, including both genders and weight of 17-22 kg. Due to the repeated collection of lavage fluid and the volume of required biological samples, dog was used as an animal model in the present experimental study. In this study, montelukast (Airokast tablet) produced by Abidi Company was used and fed directly to the animal through the gastrostomy tube. Lipopolysaccharide (Escherichia coli LPS, serotype O26:B6 commercially available as a lyophilized powder purchased from Sigma, USA) in a dose of (0.1 µg/kg) diluted in saline was intravenously injected to induce pneumonia in dogs (15).

The dogs were randomly divided into four groups: in the first group, bronchoalveolar lavage and blood sampling were performed, and after half an hour, montelukast (1 mg/kg) was fed according to the study of Mandelker in 2000, and one hour later, lavage was performed again and blood sampling was performed (16). In the second group, bronchoalveolar lavage and blood sampling were first performed and after one hour, Escherichia coli lipopolysaccharide with a dose of 0.1 µg/kg was injected intravenously according to the study of Numata et al. (17) and half an hour later, lavage and blood sampling were performed again. In the third group, lavage and blood sampling were performed first, Montelukast was fed with the above dose and one hour later, Escherichia coli lipopolysaccharide similar to second group was injected and half an hour later, lavage and blood sampling were performed again. The fourth group was similar to the third group, except that after lavage and blood sampling, first Escherichia coli lipopolysaccharide was injected and half an hour later,

[DOR: 20.1001.1.15614107.1400.23.1.33.2]
[DOI: 10.22088/jbums.23.1.229]

montelukast was fed. Then, one hour later, lavage and blood sampling were performed. After collecting bronchoalveolar fluid, the animal was first anesthetized with a mixture of ketamine and propofol, and a suitable tracheal tube was inserted into their airway. 20 ml of warm saline entered the respiratory tract through a catheter and was immediately lavaged. This step can be repeated up to three times if necessary to collect foamy liquid that indicates the presence of surfactant (18, 19). The obtained lavage liquid was transferred to a tube containing EDTA and after centrifugation, the upper part of the liquid was separated. Blood samples were taken from the dogs and serum was isolated and the samples were stored at -80 °C.

Evaluation of oxidative stress markers (MDA, GPX, catalase and total antioxidant capacity): Catalase (CAT) and malondialdehyde (MDA) levels, glutathione peroxidase (GPX) activity and total antioxidant capacity in serum and lavage fluid samples were determined with available commercial kits (Navand Salamat, Urmia, Iran) and using ELISA reader according to the manufacturer's instructions. Independent variables in this study included Escherichia coli lipopolysaccharides and montelukast as independent variables and oxidative stress factors (MDA, GPX, catalase and total antioxidant capacity) and time of administration as dependent factors.

Statistical analysis: To compare data, One-Way ANOVA and Tukey post Hoc test (for comparison between groups at one time) and paired t-student test (for comparison between the first and second time in each group) and SPSS version 19 (IBM, USA) were used and $p \leq 0.05$ was considered significant.

Results

A) Catalase in lavage fluid: With the use of montelukast alone, the amount of lavage catalase increased from 0.019±0.001 to 0.021±0.001 µmol/ml/min (p= 0.003). LPS severely and significantly decreased the levels of catalase (from 0.018±0.001 to 0.007±0.001 µmol) (p= 0.0001). Consumption of montelukast after LPS was able to prevent the severity of catalase reduction in lavage fluid (reached 0.016±0.001 µmol) and was significant compared with the other three groups (p= 0.001) (Figure 1).

Serum catalase: Consumption of montelukast alone significantly increased serum catalase (p= 0.001) in the second time (from 0.003±0.001 to 0.005±0.0009 µmol). Induction of LPS caused a severe and significant decrease (p< 0.001) in serum catalase compared to the first time in the same group (from 0.009±0.0004 to 0.002±0.0007 µmol). Consumption of montelukast had a supportive effect on catalase activity (Figure 2).

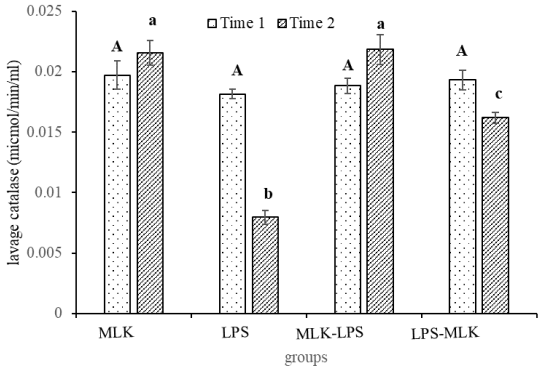

Figure 1. Mean±standard error of catalase activity in lavage fluid in different groups. (Different letters indicate a significant difference between groups with p≤ 0.05).

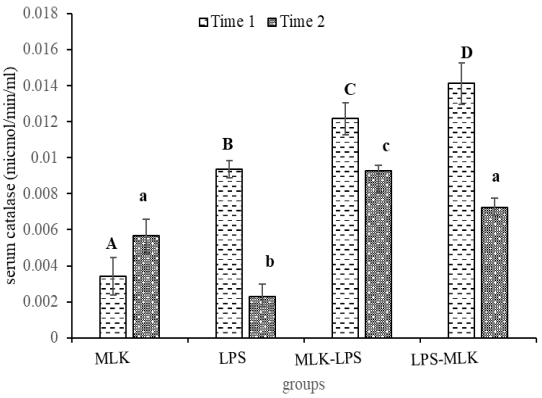

Figure 2. Mean±standard error of serum catalase activity in different groups. (Different letters indicate a significant difference between the groups with p≤ 0.05. The second time was indicated in lower case. The mean serum catalase was significantly different between the first time of each group and the second time of the same group).

B) Malondialdehyde: Consumption of montelukast alone significantly reduced the amount of malondialdehyde at the second time (from 15.7±0.8 to 12.4±1.5 µmol) (p= 0.019). With LPS injection, the amount of malondialdehyde in the lavage fluid increased severely and this increase was about 13 times more (from 10.5±1.3 to 139.8±16.8 µmol), showing a significant difference with the first time of the same group and the first and second times of the other groups (p< 0.0001). With the use of montelukast, the intensity of malondialdehyde change in lavage fluid was much less (p< 0.001) (Figure 3). Serum malondialdehyde changes were similar to those in lavage fluid and LPS

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caused a severe and significant increase in serum malondialdehyde levels, but this change was controlled by administration of montelukast (p< 0.001).

C) Glutathione peroxidase: Consumption of montelukast alone significantly increased the activity of glutathione peroxidase enzyme in the second time (from 195±10.96 to 252±8.02 nmol) (p= 0.001). The activity of glutathione peroxidase enzyme in lavage fluid decreased severely with LPS injection (from 259±13.44 to 76.5±9.24 nmol), which was significantly different from the first time of this group (p< 0.001). The amount of lavage fluid glutathione peroxidase was significantly reduced by montelukast (p= 0.001) (Figure 4). Changes in serum glutathione peroxidase are approximately similar to those in lavage fluid.

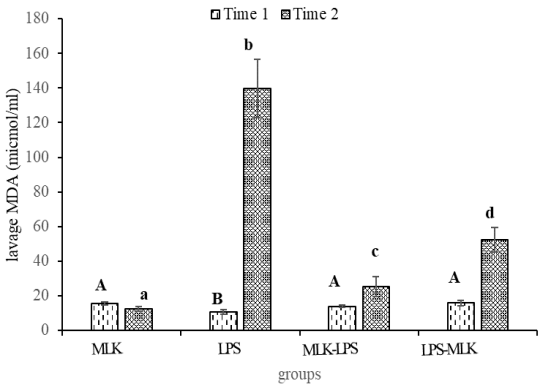

Figure 3. Mean±standard error of the amount of malondialdehyde in lavage fluid in different groups. (Different letters indicate a significant difference between the groups with p≤ 0.05. The second time was indicated in lower case. The mean malondialdehyde in the lavage fluid was significantly different between the first time of each group and the second time of the same group).

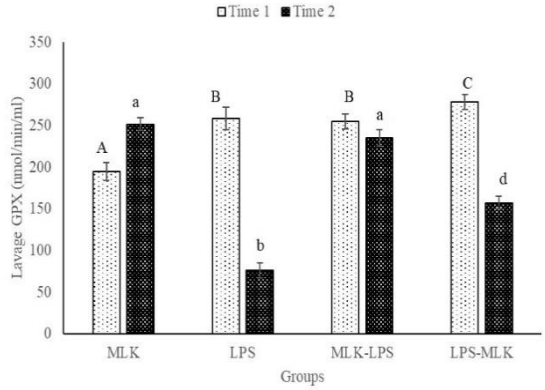

Figure 4. Mean±standard error of glutathione peroxidase activity in lavage fluid in different groups. (Different letters indicate a significant difference between groups with p≤ 0.05. The second time were marked with lowercase letters).

D) Total antioxidant level: LPS in the second time severely reduced the total antioxidant level in lavage fluid (from 0.41±0.02 to 0.04±0.01 nmol) and this difference was significant compared with other groups (p< 0.001). Consumption of montelukast before and after LPS prevented the severe decrease in total antioxidant levels in the lavage fluid (Figure 5). The rate of change in serum antioxidant levels was almost similar to that in lavage fluid.

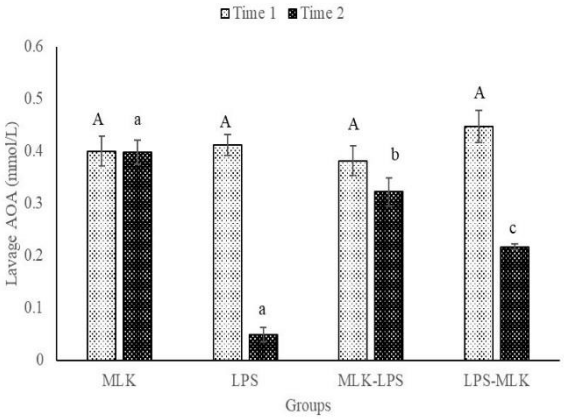

Figure 5. Mean±standard error of total antioxidant level in lavage fluid in different groups. (Different letters indicate a significant difference between groups with p≤ 0.05. The second significant time was marked with lowercase letters).

Discussion

In this study, LPS injection decreased catalase, glutathione peroxidase and total antioxidant levels in serum and bronchoalveolar lavage fluid and increased malondialdehyde levels. Consumption of montelukast was able to prevent these changes, although administration of montelukast before LPS injection generally had a better effect. Oxidative stress occurs due to an imbalance between oxidant-antioxidant systems, which can be due to an increase in free radicals and a decrease in antioxidant activity (20). Malondialdehyde (MDA) is a sign of lipid peroxidation and is one of the most important biomarkers of oxidative stress (21). Glutathione plays an important role in maintaining protein and lipid integrity and provides major protection against oxidative damage (22). Weifeng et al., by injecting lipopolysaccharide (E. coli O55: B5, 5mg/Kg) LPS intratracheally in mice and causing acute lung damage, examined changes in pulmonary oxidative stress factors. They stated that 2 hours after lipopolysaccharide inoculation, the amount of malondialdehyde in lung tissue increased and also the

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total antioxidant capacity decreased and the transcriptional level of enzymes involved in antioxidant defense increased (23). The result of the present study, which demonstrates the effect of lipopolysaccharide on oxidative stress, even for a short time, is consistent with the above study.

In the present study, montelukast had a significant effect on antioxidant defense and reduction of oxidative stress, especially before LPS injection. Montelukast reduces inflammation induced by eosinophil infiltration (24). Khodir et al. reported that LPS significantly increased lung and kidney MDA levels and decreased glutathione. Montelukast significantly decreased MDA and increased renal glutathione (25). The result of our study is consistent with the above study and shows the antioxidant effects of montelukast against oxidative stress induced by lipopolysaccharide.

In another study, Coskun et al. examined the protective effects of montelukast on tissue damage induced by sepsis in vital organs such as the heart, liver, kidneys, and especially the lungs in rats. Serum levels of cytokines in the sepsis group increased compared with the control (TNF-α up to 10 folds and IL-6 up to 7 folds), which decreased significantly with administration of montelukast in septic rats. Montelukast was effective in reducing the levels of oxidative parameters of lung, liver, heart and kidney tissue. The amount of glutathione and superoxide dismutase activity in lung, liver and kidney tissues was increased by montelukast. In this study, lung and kidney tissue had the highest level of protection by montelukast in conditions of sepsis (12).

Considering that they play an important role in causing inflammation and oxidative stress in sepsis and bacterial infections, especially gram-negative bacteria, and since the content of the bacteria, especially lipopolysaccharide, is involved in this issue, therefore, the consumption of montelukast could have a protective effect on many tissues of the body, including the lungs, which is consistent with the results of the present study. Although the type of studied animal was different, the efficacy of montelukast was similar in both animal models. Şener et al. also showed that montelukast improves hepatic and intestinal damage in experimental septic conditions (9). In the study by Coskun et al., it was reported that montelukast can prevent the accumulation of neutrophils in tissues by reducing oxidative stress and protecting membrane permeability (12). In addition, El- shafaei et al. found that montelukast, like melatonin, had antioxidant effects against cisplatin-induced oxidative stress in rats (26).

There is another report by Khodir et al. regarding the protective effect of montelukast due to its antioxidant and anti-inflammatory properties against LPS-induced heart damage (27). Although the animal species and tissues studied in the above study were different from the present study, the results were consistent with our study in confirming the antioxidant properties of montelukast. There are other studies in line with the present study to confirm the antioxidant effects of montelukast, including Hareedy et al., who found that montelukast has protective effects against simvastatin-induced liver and muscle damage (28).

In this experimental study, it was observed that LPS injection decreased the activity of catalase, glutathione peroxidase and total antioxidant levels in serum and bronchoalveolar lavage fluid of dog and increased the amount of malondialdehyde, but montelukast was able to correct the changes in oxidative stress factors.

Acknowledgment

We would like to thank the Vice Chancellor for Research of Shahid Chamran University of Ahvaz for the financial support of this research.

[DOR: 20.1001.1.15614107.1400.23.1.33.2]
[DOI: 10.22088/jbums.23.1.229]

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