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BML-111 attenuates acute lung injury in endotoxemic mice

Min Tang, MD,[a,b] *Lin Chen, MD,*[a,b] *Bo Li, MD,*[a,b] *Yaxin Wang, MD,*[a,b] *Shengnan Li, MD,*[a,b] *Aiqing Wen, MD,*[c] *Shanglong Yao, MD, PhD,*[a,b] *and You Shang, MD, PhD*[a,b,*]

[a] *Department of Critical Care Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China*

[b] *Institute of Anesthesia and Critical Care, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China*

[c] *Department of Blood Transfusion, Research Institute of Surgery, Daping Hospital, Third Military Medical University, Chongqing, China*

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ABSTRACT

Background: BML-111 is a lipoxin receptor agonist that has protective effects in various lung injury models. We tried to elucidate whether BML-111 could mitigate lung injury in a mouse model of endotoxemia and endothelial hyperpermeability *in vitro*.

Methods: The effect of BML-111 on lung injury was evaluated using C57BL/6 mice and human umbilical vein endothelial cells (HUVECs). Male C57BL/6 mice were intraperitoneally injected with normal saline, BML-111, and/or the lipoxin receptor antagonist Boc-2. Then, either lipopolysaccharide (LPS) or normal saline was given intraperitoneally. Lung injury was assessed by a pathohistologic examination for neutrophil infiltration, pulmonary endothelial permeability, and inflammatory cytokines in lung tissue and bronchoalveolar lavage fluid. HUVECs were treated with or without BML-111 before incubation with LPS for 24 h. Boc-2 was also tested as a novel inhibitor of BML-111. A Transwell assay was used to evaluate the permeability of HUVECs. Junction protein expression was also assessed.

Results: BML-111 significantly improved the mouse survival rate, reduced body weight loss, attenuated the pulmonary pathologic changes, inhibited neutrophil infiltration and proinflammatory cytokine production, and mitigated endothelial hyperpermeability. The decreased expression of junction proteins induced by LPS in lung tissue and endothelial cells were upregulated by BML-111. In addition, BML-111 inhibited the activation of the Akt, ERK1/2, and p38 MAPK signaling pathways. However, the beneficial effects of BML-111 were abolished by Boc-2.

Conclusions: BML-111 attenuated lung injury in endotoxemic mice and mitigated endothelial hyperpermeability by upregulating the expression of junction proteins.

M.T. and L.C. made equal contribution to this work.

* *Corresponding author.* Department of Critical Care Medicine, Institute of Anesthesia and Critical Care, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1277, Jiefang Ave, Wuhan, Hubei 430022, China. Tel.: +86 15972127819; fax: +86 2785351606.

E-mail address: you_shang@yahoo.com (Y. Shang).

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1. Introduction

Acute lung injury (ALI), or acute respiratory distress syndrome, is a clinical syndrome associated with respiratory dysfunction. It is a complication of sepsis and has a ~50% mortality rate [1]. The administration of lipopolysaccharide (LPS), a gram-negative bacterial endotoxin, has been used as a model of sepsis-related lung injury in different studies [2,3].

The endothelium plays a critical role in the pathophysiology of systemic inflammation, regulating cellular trafficking, maintaining blood fluidity, contributing to the local balance in pro- and anti-inflammatory mediators, participating in generation of new blood vessels, and undergoing programmed cell death [4]. The importance of endothelial barriers, which separate blood and air in the lung and increase vascular permeability in pulmonary edema, in ALI has been well established [5]. Endothelial cell junctions present a particularly complex network of adhesion proteins that are linked to the intracellular cytoskeleton and signaling partners. These proteins are organized into distinct structures called tight junctions (TJs) and adherens junctions (AJs) [6]. Defects in the organization of endothelial cell junctions are associated with many disease pathologies, such as vascular malformations, hemorrhage, and tissue edema. Thus, maintaining the endothelial cell junctions would be an effective therapeutic strategy for ALI in endotoxemic mice.

Lipoxins (LXs) are the first recognized lipid mediators. They have unique structures that are derived from arachidonic acid and dual anti-inflammatory and proresolution activities [7]. Proresolving mediators, such as LX and maresin 1, have been demonstrated to have protective effects in many inflammatory and organ-specific disease models, such as brain ischemia–reperfusion injury [8,9], colitis [10], ALI [11], asthma [12], and so on. BML-111 (Fig. 1) is a stable biosynthesized agonist of the LXs receptor (formyl peptide receptor-2) [13] and displays proresolving and anti-inflammatory effects in carbon tetrachloride–induced liver injury [14], hemorrhagic shock–induced ALI [15], and ventilator-induced lung injury [16].

However, the protective effects of BML-111 in lung injury in a mouse model of endotoxemia remain unknown. To elucidate these effects, we carried out the present study with the hypothesis that BML-111 would mitigate lung injury in endotoxemic mice and alleviate endothelial hyperpermeability *in vitro*.

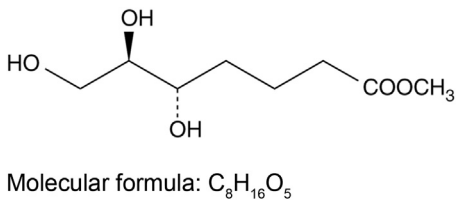

Fig. 1 – The pictorial of BML-111 and its molecular formula.

2. Materials and methods

2.1. Animals

Two hundred male C57BL/6 mice (8–10-wk-old, 20–25 g) were purchased from Wuhan University Laboratory Center (Wuhan, China). All the animal experiments were approved by the Animal Care and Use Committee of Tongji Medical College of Huazhong University of Science and Technology. All the animal studies have been reported in accordance with the animal research: reporting *in vivo* experiments guidelines for reporting experiments involving animals [17]. Mice were maintained in a pathogen-free room. The animals were given standard laboratory chow and water *ad libitum* and were housed in a room with temperature between 22 and 24°C and humidity of 60%–65% with 12-h light–dark cycles.

2.2. Animal experimental procedures

Mice were randomly divided into five groups. The control group (Control) consisted of mice injected intraperitoneally (i.p.) twice, 30-min apart, with normal saline. The sham group (BML-111) consisted of mice injected i.p. with BML-111 (Cayman Chemical, Ann Arbor, MI) at a dosage of 1 mg/kg 30 min before an i.p. Natural Saline (NS) injection [15]. The LPS group (LPS) consisted of mice with an i.p. injection of NS 30 min before an i.p. LPS injection (from *Escherichia coli serotype O55:B5*; Sigma–Aldrich Co, St. Louis, MO) at a dosage of 10 mg/kg. The treatment group (BML-111 + LPS) consisted of mice injected i.p. with BML-111 30 min before an i.p. LPS injection. The antagonist group (Boc-2) consisted of mice injected i.p. with Boc-2 (GenScript, Piscataway, NJ) at a dosage of 50 μg/kg 15 min before the BML-111 i.p. injection, followed by LPS administered i.p. 30 min after BML-111. All these chemical reagents were diluted with NS to the same volume (0.1 mL per mouse).

The mice were sacrificed by an overdose of sodium pentobarbital 24 h after LPS administration.

2.3. Survival rate and body weight changes

Sixteen mice in each group were closely observed for mortality for 7 d. The body weights were recorded at 0 and 24 h after the LPS challenge to calculate the body weight loss rate.

2.4. Histologic analysis of lung tissues

The lower right lung lobes were inflated to 15 cm of H_2O with 4% paraformaldehyde and removed for paraffin embedding. Sections were stained with hematoxylin and eosin. Under light microscope, histologic changes were evaluated. In ALI, the changes included alveolar congestion, hemorrhage, neutrophil infiltration, and thickness of alveolar wall and/or hyaline membrane formation. Each histologic change was scored on a scale of 0 = minimal (little) damage to 4 = maximal damage by a histologist who was blinded to the treatment groups. A total lung injury score was calculated as the sum of the four items, as previously reported [18].