Anti-Inflammatory Activity of Antimicrobial Peptide Allomyrinasin Derived from the Dynastid Beetle, *Allomyrina dichotoma*

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Received: September 17, 2018
Revised: March 4, 2019
Accepted: March 21, 2019

First published online
March 28, 2019

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pISSN 1017-7825, eISSN 1738-8872

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In a previous work, we performed *de novo* RNA sequencing of *Allomyrina dichotoma* using next generation sequencing and identified several antimicrobial peptide candidates based on transcriptome analysis. Among them, a cationic antimicrobial peptide, allomyrinasin, was selected bioinformatically based on its physicochemical properties. Here, we assessed the antimicrobial and anti-inflammatory activities of allomyrinasin against microorganisms and mouse macrophage Raw264.7 cells. Allomyrinasin showed antimicrobial activities against various microbes and decreased the nitric oxide production of the lipopolysaccharide-induced Raw264.7 cells. Furthermore, quantitative RT-PCR and ELISA revealed that allomyrinasin reduced cytokine expression levels in the Raw264.7 cells. We also identified inducible nitric oxide synthase, cyclooxygenase-2 expression, and PGE₂ production through western blot analysis and ELISA. We confirmed that allomyrinasin bound to bacterial cell membranes via a specific interaction with lipopolysaccharides. Taken together, these data indicate that allomyrinasin has antimicrobial and anti-inflammatory activities as exemplified in lipopolysaccharide-induced Raw264.7 cells. We have provided a potentially useful antimicrobial peptide candidate that has both antimicrobial and anti-inflammatory activities.

Keywords: Antimicrobial peptide, anti-inflammatory activity, inflammation, lipopolysaccharide, *Allomyrina dichotoma*

Introduction

Allomyrina dichotoma, a species of beetle, has been used in traditional medicine in Asian countries for the treatment of various diseases. For instance, it is known that *A. dichotoma* larvae have anti-neoplastic, anti-obesity, anti-Alzheimer and anti-oxidant activities [1–10]. However, the pharmacological efficacy and useful medicinal ingredients of this beetle have not been thoroughly determined. Most studies that have been reported used extracts of *A. dichotoma* larvae and only a few compounds of the beetle were reported [11]. In addition, only three antibacterial peptides (defensin) and proteins (coleopteracin A and B) have been purified from the immunized hemolymph of *A. dichotoma* larvae [12, 13].

Antimicrobial peptides (AMPs) have an important role in

innate immunity against invading pathogens for the maintenance of homeostasis [14, 15]. The physicochemical properties of AMPs and their mechanisms of action have been well studied [16]. AMPs encounter surface molecules of bacterial membranes and interact with the negatively charged surface molecules to produce antibacterial activity. For instance, lipopolysaccharide (LPS) and lipid A in gram-negative bacteria and peptidoglycan (PGN) and lipoteichoic acid (LTA) in gram-positive bacteria are targets for the initial interaction. These targets are also known as pathogen-associated molecular patterns (PAMPs).

LPS, also known as endotoxin, is a principal component of the outer membrane of gram-negative bacteria and is known to be a mediator of sepsis and septic shock. Sepsis induces the expression of several proinflammatory cytokines, which causes cell damage and tissue injury [17,

18]. In this context, AMPs can bind and neutralize LPS and prevent LPS-induced cytokines in macrophages [19]. Neutralization of LPS also inhibits sepsis and septic shock in mouse models [20]. Here, we demonstrate the antimicrobial and anti-inflammatory activities of allomyrinasin, which are primarily due to the peptide's interaction with LPS on the cell surface.

Materials and Methods

Peptide

Allomyrinasin was synthesized using the solid-phase peptide synthesis method by Anygen Co., Ltd. (Korea). The peptide was dissolved in acidified distilled water (0.01% acetic acid) and stored at -20°C until use.

Antimicrobial Assay

The antimicrobial activity of allomyrinasin was tested through radial diffusion assay [21]. Stock peptide solution was prepared in acidified distilled water (0.01% acetic acid) and 5 μl samples were introduced as a series of five serial two-fold dilutions. These concentrations ranged from 25 to 200 μg of peptide/ml and were loaded into the wells (3 mm in diameter) in the underlay, in which washed mid-logarithmic phase bacteria and yeast were trapped. The underlay agar consisted of 9 mM sodium phosphate, 1 mM sodium citrate buffer, 1% (w/v) agarose (Sigma, USA), and 0.3 mg of tryptic soy broth (TSB; Difco, USA). After incubation at 37°C for 3 h, a 10-ml overlay agar containing 1% agarose and 6% TSB was poured onto the underlay agar. After the plates were incubated overnight, the diameters of clearing zones, which indicate antimicrobial activity, were plotted against the peptide concentrations.

Cell Culture

Raw264.7 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin G (100 U/ml), and streptomycin (100 μg /ml) (Invitrogen, USA). Cells were cultured at 37°C in a humidified incubator with 5% CO_2 .

MTS Assay

Raw264.7 cells plated in 96-well plates (2×10^4 cells/well) were treated with allomyrinasin at varying concentrations (25, 50, 100, and 200 μg /ml). After incubation for 24 h, the viability of the cells was assessed using the Cell Titer 96 Aqueous One Solution Cell Proliferation Assay according to the manufacturer's protocol (Promega, USA). The optical density at 490 nm was measured with a microplate reader (Beckman DTX 8800 Multimode Detector, USA).

Nitric Oxide (NO) Assay

Raw264.7 cells were seeded at 1×10^6 cells/ml in a 6-well culture plate in assay medium (DMEM) and were treated with different doses of allomyrinasin for 1 h. For the positive control

setup, 1 μg of LPS solution was added to the well with peptide-treated wells. For the negative control setup, an equal volume of distilled water was added to the well. The plate was incubated for 24 h in a CO_2 incubator and then culture supernatant was collected from each of the wells. For the NO assay, 100 μl of sample was added to each well of the 96-well culture plate and was incubated with 50 μl of N1 buffer (substrate solution) for 5 min at room temperature. After incubation, 50 μl of N2 buffer (coloring solution) was added to the wells and the mixture was incubated for 10 min at room temperature. The absorbance of each well was subsequently measured using a microplate reader at 550 nm. The percent of NO production was calculated based on the LPS-treated sample as the maximum NO level.

RNA Isolation and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was isolated from Raw264.7 cells using the TRIzol reagent according to the manufacturer's instructions (Ambion, USA). cDNA was synthesized at 37°C for 1 h using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). The resulting cDNA was used to amplify genes using primers specific to mouse tumor necrosis factor- α (*TNF- α*) (forward 5'-ATGAGAAGTCCCCAAATGGC-3', and reverse 5'-CTCCACTTGGTGGTTTGCTA-3'), interleukin-6 (*IL-6*) (forward 5'-GAGGATACCACTCCCAACAGACC-3', and reverse 5'-AAG TGCATCATCGTTGTTTCATACA-3'), *IL-1 β* (forward 5'-CCTTCC AGGATGAGGACATGA-3', and reverse 5'-TGAGTCACAGAG GATGGGCTC-3'), inducible nitric oxide synthase (*iNOS*) (forward 5'-CAGCACAGGAAATGTTTCAGC-3', and reverse 5'-TAGCCAGCGTACCGGATGA-3'), and cyclooxygenase-2 (*COX-2*) (forward 5'-CAGACAACATAAACTGCGCCTT-3', and reverse 5'-GATACACCTCTCCACCAATGACC-3'). Real-time PCR was performed using an ABI 7500 Real Time PCR System (PE Applied Biosystems, USA) with Power SYBR Green PCR Master Mix (Applied Biosystems). The cycling conditions consisted of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min.

Enzyme-Linked Immunosorbent Assay (ELISA)

To measure the production of IL-6 and IL-1 β , two proinflammatory cytokines known to reflect macrophage activation, and prostaglandin E_2 (PGE_2) production, Raw264.7 cells (1×10^6 cells/well) were treated with medium alone, LPS alone, or allomyrinasin with LPS for 24 h. Supernatants were collected, and the concentrations of IL-6 and IL-1 β were measured using ELISA (Thermo Fisher Scientific, USA) and the concentrations of PGE_2 were measured using ELISA (R&D Systems, USA).

Western Blot Analysis

Raw264.7 cells were seeded in 6-well tissue culture plates (1×10^6 cells per well) and pretreated with various concentrations of allomyrinasin (25, 50, 100, and 200 μg /ml) for 1 h. They were further incubated with medium alone, LPS alone, or allomyrinasin with LPS for 30 min or 24 h. After incubation for the indicated

time, cells were washed with cold phosphate-buffered saline (PBS) and their proteins were extracted in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer. The protein samples were separated via 10% SDS-PAGE, and the gels were transferred electrically to nitrocellulose membranes. Membranes were blocked in 5% skim milk (w/v) in Tris-buffered saline (TBS; pH 7.5) for 1 h at room temperature, and then incubated overnight at 4°C with antibodies against β -actin, iNOS, COX-2, anti-phospho and total-p44/p42 MAPK, anti-phospho and total-p38 MAPK, anti-phospho and total JNK, and I κ B (all antibodies from Cell Signaling Technology, USA). After two washings with TBS containing 0.05% Tween-20 (TBST), the antigens were detected with HRP-conjugated secondary antibody (Promega, USA), and the signals were visualized on FluorChem (Alpha Innotech Corporation, USA) using Western Lightning Plus (PerkinElmer, USA).

Allomyrinasin Binding Assay

The binding of allomyrinasin to the surface of microbes was examined by assessing the effect of cell membrane components on the antimicrobial activity of allomyrinasin against *Escherichia coli* through a radial diffusion assay and *E. coli*-binding assay. For the radial diffusion assay, 200 μ g/ml of allomyrinasin was incubated with varying concentrations of LPS for 10 min at 37°C in 10 mM sodium phosphate buffer (pH 7.4). Then, 5 μ l samples of each mixture were loaded into wells (3 mm diameter) that had been punched into the underlay agar containing washed mid-logarithmic *E. coli* (4×10^6 colony-forming units). After incubation at 37°C for 3 h, a 10-ml overlay agar containing 1% agarose and 6% TSB was poured onto the underlay agar. After the plates were incubated overnight, the diameters of clearing zones were plotted. In case of *E. coli*-binding assay, *E. coli* was cultivated overnight in TSB at 37°C and fixed for 20 min using paraformaldehyde (Electron Microscopy Sciences, UK). Paraformaldehyde-fixed *E. coli* cells were washed three times with a sterile 10 mM sodium phosphate buffer, pH 7.4. Fixed *E. coli* (about 10^9 cells) was re-suspended in 10 mM sodium phosphate buffer containing allomyrinasin at a concentration of 100 μ g/ml. The mixture was incubated at room temperature for 1 h in a shaking incubator and then subjected to centrifugation at 2,000 g for 10 min. After the sediment was washed three times with 10 mM sodium phosphate buffer, it was re-suspended in 0.05 M sodium acetate buffer (pH 3.6) containing 0.5 M NaCl and incubated at room temperature for 30 min in a shaking incubator. After centrifugation at 2,000 g for 10 min, *E. coli*-binding peptide detached from the cell walls of *E. coli* was removed as supernatant. The supernatant was directly subjected to a C18 UPLC column (Halo ES-C18). Peptide eluted as a peak on the UPLC profile and compared to the retention time of the allomyrinasin peak.

Statistical Analysis

Data are presented as mean $\pm$ standard deviation (SD) of at least three independent experiments. Differences among groups were

Fig. 1. Antimicrobial activities of allomyrinasin against microorganisms determined through radial diffusion assay. Peptide concentration (x-axis) was plotted against the diameter of the microbial growth inhibition zone (y-axis) after incubation for 12 h, and is expressed in units (1 mm = 10 units). Data shown are means $\pm$ standard deviations (SD) of triplicate experiments.

evaluated by Duncan post-hoc ANOVA analysis and considered statistically significant at $p < 0.05$.

Results and Discussion

Antimicrobial Activity of Peptides

In a previous study, we analyzed the transcriptome of *A. dichotoma* and identified AMP candidates using bioinformatics tools (unpublished data). Allomyrinasin (sequence: AAVTRRILCWFA-NH₂) was one of the identified candidates and its antimicrobial activity was tested against several microorganisms (Fig. 1). Allomyrinasin revealed potent antimicrobial activities toward *E. coli* and *Staphylococcus aureus*, while the peptide had relatively weak antimicrobial activities against *Propionibacterium acnes*, *S. epidermidis*, and *Candida albicans*. Allomyrinasin is a cationic peptide with a net positive charge (+3) at neutral pH. These kinds of peptides are known as cationic antimicrobial peptides (CAPs) and can interact with and neutralize LPS. Recently, LPS neutralization has been used as a therapeutic approach to prevent septic shock [22]. Thus, CAPs are a promising new agent for the treatment of sepsis and septic shock. For instance, polymyxin B is the most well-studied cationic peptide in terms of LPS binding and detoxification [23]. Here, we investigated the anti-

Fig. 2. Cell viability and inhibition of NO production in Raw264.7 cells after allomyrinasin treatment.

(A) Cell viability was measured through the MTS assay after 24 h incubation with the indicated concentrations of the peptides. (B) Cells were pretreated with allomyrinasin for 1 h prior to incubation with LPS for 24 h. The NO in the culture media was measured with the Griess reagent. Data shown are means $\pm$ SD of triplicate experiments. Statistical analyses were performed as described. [#] $P < 0.05$ in comparison with control group. ^{***} $P < 0.001$, ^{**} $P < 0.01$, ^{*} $P < 0.05$, compared to the LPS-treated group. CTR, control; LPS, lipopolysaccharide.

microbial and anti-inflammatory activities of allomyrinasin due to a possible interaction between the peptide and LPS.

Allomyrinasin Inhibits NO Production in LPS-Stimulated Raw264.7 Cells

It has been reported that several antimicrobial peptides showed anti-inflammatory activities in LPS-induced macrophages and animal models [24]. These peptides are mostly cationic amphipathic peptides, and the positive charge of these peptides is important for electrostatic

interactions with membrane surface molecules of bacteria [25]. We tested for possible a cytotoxic effect of allomyrinasin on the viability of Raw264.7 cells. Raw264.7 cells were treated with various concentrations of allomyrinasin (25, 50, 100, and 200 µg/ml) for 24 h, and cell viability was measured through MTS assay. Allomyrinasin did not decrease the viability of the Raw264.7 cells even at the highest concentration (Fig. 2A). We then applied the same concentration range of allomyrinasin for the following experiments. We subsequently investigated the effect of allomyrinasin on NO production in LPS-induced Raw264.7 cells and found that NO production induced by LPS was reduced after treatment with allomyrinasin at over 100 µg/ml (Fig. 2B).

Allomyrinasin Reduces the Expression of Proinflammatory Cytokines in LPS-Stimulated Raw264.7 Cells

We determined the effect of allomyrinasin on the expression of proinflammatory cytokines (TNF- α , IL-6, and IL-1 β) of Raw264.7 cells using qRT-PCR and ELISA. The transcriptional expression levels of IL-6 and IL-1 β in Raw264.7 cells upon allomyrinasin treatment decreased in a dose-dependent manner (Fig. 3A). However, allomyrinasin did not inhibit TNF- α expression in LPS-stimulated Raw264.7 cells. The expression levels of IL-6 and IL-1 β are consistent with the results of the NO assay, suggesting a specific anti-inflammatory activity of allomyrinasin on the Raw264.7 cells. In addition, we confirmed the production of the cytokines at the protein level from the Raw264.7 cells upon allomyrinasin treatment. Results showed that the release of cytokines was reduced by allomyrinasin treatment at doses of 100 µg/ml and/or 200 µg/ml (Fig. 3B). These results indicate that allomyrinasin can affect the inflammatory responses of LPS-induced Raw264.7 cells. Previously, our group reported an insect defensin, named coprisin, identified from the dung beetle (*Copris tripartitus*), and analyzed its three-dimensional structure in aqueous solution by nuclear magnetic resonance spectroscopy [26]. Based on the result, we determined an α -helical region of the peptide and modified it by truncation and substitution. As a result, we designed a coprisin analog, named CopA3, consisting of a 9-mer peptide (LLCIALRKK). Then, we prepared a dimeric form of CopA3 through a disulfide linkage and investigated its anti-inflammatory activities in LPS-induced macrophages. Coprisin and dimer CopA3 inhibited the expression of proinflammatory cytokines in LPS-activated Raw264.7 cells and/or mouse peritoneal macrophages [26, 27]. Allomyrinasin and the aforementioned peptides are CAPs, which share common features such as a

Fig. 3. Inhibitory effects of allomyrinasin on the production of proinflammatory cytokines in LPS-induced Raw264.7 cells.

(A) Raw264.7 cells were pretreated with allomyrinasin for 1 h prior to incubation with LPS. RNA was isolated at 12 h after LPS treatment. The levels of *IL-6* and *IL-1β* mRNA were determined through quantitative real-time-PCR. The data were normalized to *Gapdh* mRNA level. (B) Raw264.7 cells were pretreated with allomyrinasin for 1 h prior to incubation with LPS for 24 h. IL-6 and IL-1β levels in the culture media were measured through ELISA. Data shown are means ± SD of triplicate experiments. [#]*P* < 0.05 in comparison with control group. ^{***}*P* < 0.001, ^{**}*P* < 0.01, ^{*}*P* < 0.05 in comparison with LPS group. CTR, control; LPS, lipopolysaccharide.

basic pH and a positive net charge with high isoelectric point value. The cationic nature of these peptides is important for their interaction with anionic cell surface molecules of bacteria.

Allomyrinasin Inhibits iNOS, COX-2 Expression and PGE₂ Production in LPS-Stimulated Raw264.7 Cells

We also analyzed the expression of inflammatory mediators upon allomyrinasin treatment. The transcriptional expression level of iNOS and COX-2 in the Raw264.7 cells decreased in a dose-dependent manner (Fig. 4A). In addition, we also analyzed the protein expression of these inflammatory mediators through western blot analysis and revealed that the expression of iNOS and COX-2 increased after LPS treatment, but was suppressed upon allomyrinasin treatment

at a concentration of 200 µg/ml (Fig. 4B). Moreover it is known that COX-2 induces PGE₂ production in activated macrophages during inflammatory reaction. Thus we investigated the production of PGE₂ using ELISA. As a result, PGE₂ induced by LPS treatment, but inhibited upon allomyrinasin treatment over 100 µg/ml (Fig. 4C). The inhibitory effect of allomyrinasin on PGE₂ production showed similar aspect with COX-2 expression.

Specific Binding of Allomyrinasin to LPS of Bacterial Cell Membranes

The specific binding of allomyrinasin to the microbial surface was confirmed to be mediated through a microbial cell membrane component. Two hundred µg/ml of allomyrinasin was incubated with varying concentrations

Fig. 4. Inhibitory effects of allomyrinasin on iNOS, COX-2 expression and PGE₂ production in LPS-induced Raw264.7 cells.

(A) Raw264.7 cells were pretreated with allomyrinasin for 1 h prior to incubation with LPS. RNA was isolated 12 h after LPS treatment. The levels of iNOS and COX-2 mRNA were determined by qRT-PCR. The data were normalized to *Gapdh* mRNA levels. (B) Raw264.7 cells were pretreated with allomyrinasin for 1 h prior to incubation with LPS. Protein was isolated 24 h after LPS treatment. The protein expression levels of iNOS and COX-2 were determined through western blot analysis. The data were normalized to β-actin protein levels. (C) Raw264.7 cells were pretreated with allomyrinasin for 1 h prior to incubation with LPS for 24 h. PGE₂ levels in the culture media were measured through ELISA. Data shown are means ± SD of triplicate experiments and are representative of three independent experiments. #*P* < 0.05 in comparison with control group. ***P < 0.001, **p < 0.01, *p < 0.05 in comparison with LPS group. CTR, control; LPS, lipopolysaccharide.

of LPS and the mixture was examined for antimicrobial activity against *E. coli* through a radial diffusion assay (Fig. 5A). Allomyrinasin clearly formed clearing zones against *E. coli* in an LPS concentration-dependent manner. The results indicated that LPS can interfere with the interaction between allomyrinasin and the *E. coli* cell surface. In addition, UPLC performed with peptide detached from the cell wall of paraformaldehyde-fixed *E. coli* generated one major peak (Fig. 5B). Fig. 5B shows the UPLC profiles and allomyrinasin was eluted with a gradient of 20–60% acetonitrile. Allomyrinasin as a standard was eluted at 36% acetonitrile (upper panel) and the detached peptide from the *E. coli* was eluted at the same acetonitrile concentration

(lower panel). From UPLC analysis, we confirmed the detached peptide is eluted at the same retention time as allomyrinasin. Thus, we surmise that allomyrinasin binds to bacteria by specifically binding to LPS. LPS binding is important to prevent sepsis and septic shock. It is known that LPS-induced inflammation in macrophages is initiated by the binding of LPS to toll-like receptor 4 (TLR4), with the subsequent signaling through nuclear factor kappa B (NF-κB) and the JAK/STAT pathway resulting in the production of proinflammatory cytokines such as IL-6 [28]. Therefore, binding of allomyrinasin to LPS may suppress the binding of LPS to TLR4, which is known as LPS detoxification and neutralization. Interestingly, it reported

Fig. 5. Specific binding of allomyrinasin to lipopolysaccharide.

(A) The radial diffusion assay was conducted by mixing varying amounts of LPS with 200 µg/ml of allomyrinasin. The mixtures of peptide with LPS were loaded into wells of the assay plate seeded with *E. coli*, which has been confirmed to be highly susceptible to allomyrinasin. The upper panel shows a photo of the gel from the radial diffusion assay. Numbers (x-axis) represent the LPS concentration (mg/ml) of the mixture loaded in the wells. Five µl of 200 µg/ml allomyrinasin was used for LPS-free control. The lower panel shows the antibacterial activity of allomyrinasin in the mixture plotted against the concentration of LPS. The diameters of the clearing zone are expressed in units (1 mm = 10 units). (B) Identification of *E. coli*-binding allomyrinasin. Allomyrinasin detached from the surface of *E. coli* was directly subjected to UPLC using C¹⁸ column (lower panel peak). Elution was performed with a linear gradient of 20–60% acetonitrile in 20 mM trifluoroacetic acid over 5 min at a flow rate of 0.8 ml/min. Allomyrinasin was re-suspended in the acidified water and re-purified by UPLC as a control (upper panel peak). Data shown are means ± SD of triplicate experiments and are representative of three independent experiments. Statistical analyses were performed as described. *** $P < 0.001$, ** $p < 0.01$, * $p < 0.05$, compared to the LPS non-treated control. LPS, lipopolysaccharide.

that CAPs block the interaction of LPS with LPS binding protein (LBP). The relative ability of CAPs to block the binding of LPS to LBP correlated with their ability to block LPS-induced TNF- α production by the Raw264.7 cells [29]. As for the anti-inflammatory effect of allomyrinasin, allomyrinasin may sequester LPS and consequently prevent LPS-mediated TLR4 signaling. Although the exact mechanism remains to be elucidated, our results suggest that allomyrinasin interacts with LPS directly.

Allomyrinasin Suppresses the LPS-Stimulated Phosphorylation of MAPKs in Raw264.7 Cells

In previous studies, it has been reported that the mitogen-activated protein kinases (MAPKs) play key regulatory roles in the production of proinflammatory mediators. Thus we investigated the effect of allomyrinasin on the LPS-induced phosphorylation of MAPKs in Raw264.7 cells via western blot analysis. Raw264.7 cells were stimulated with LPS for 30 min after allomyrinasin pretreatment for 1 h. The result showed that allomyrinasin significantly attenuated the phosphorylation of MAPKs

(Fig. 6A). In addition, the activation process of NF- κ B signaling pathways is mediated by the I κ B kinase (IKK) complex and activation of IKK by a stimulus such as LPS leads to the phosphorylation and degradation of I κ B proteins and subsequent activation of NF- κ B. Therefore, we examined the effect of allomyrinasin on I κ B degradation as an indicator of NF- κ B activation using western blot analysis (Fig. 6B). The result showed that I κ B was significantly degraded by LPS, but allomyrinasin efficiently inhibited LPS-induced I κ B degradation.

We demonstrated the antimicrobial and anti-inflammatory activities of allomyrinasin against various microbes and in LPS-induced Raw264.7 cells, respectively. Allomyrinasin showed broad-spectrum antimicrobial activity and was especially potent against *E. coli* and *S. aureus*. Moreover, allomyrinasin revealed anti-inflammatory activity in murine macrophage Raw264.7 cells. Allomyrinasin interacted with LPS, which are membrane components of gram-negative bacteria. We have provided a useful antimicrobial peptide candidate and an efficient strategy for the development of a new antimicrobial peptide.

Fig. 6. Inhibitory effect of allomyrinasin on MAPKs and NF-κB signaling pathways in Raw264.7 cells.

(A) Raw264.7 cells (1×10^6 cells/well in a 6-well plate) were incubated with the indicated concentration of allomyrinasin (μg/ml). Protein was then isolated at 30 min after LPS treatment, and phosphorylation of ERK, p38, and JNK was detected using western blot analysis. The data were normalized to total protein, respectively. (B) Degradation of IκB was detected using western blot analysis. The data were normalized to β-actin. CTR, control; LPS, lipopolysaccharide.

Acknowledgments

This work was supported by a grant from the Next-Generation BioGreen 21 Program (Project No. PJ01325601), Rural Development Administration, Republic of Korea.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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