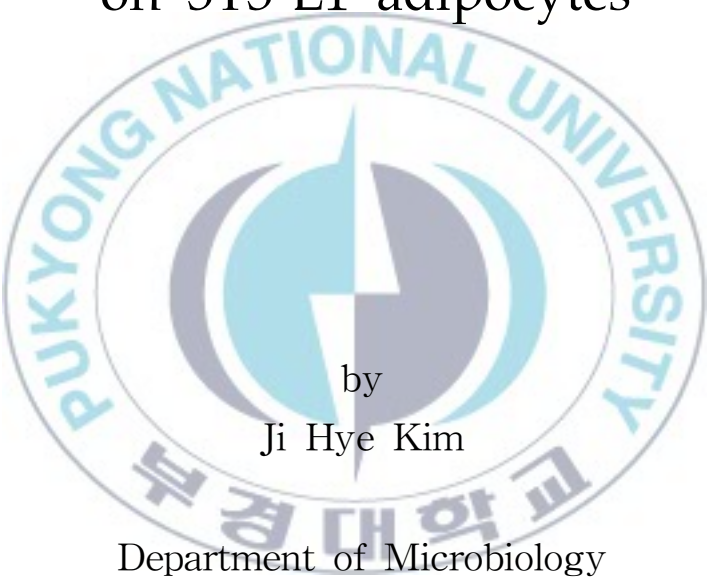
Thesis for the Degree of Master of Science

Adipogenesis inhibitory effect of Mastoparan B peptide on 3T3-L1 adipocytes



by

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February 21, 2014

Adipogenesis inhibitory effect of Mastoparan B peptide on 3T3-L1 adipocytes

(Mastoparan B 펩타이드에 의한 3T3-L1 전지방세포의 지방분화 억제 효과)

Advisor: Prof. Gun Do Kim

by

Ji Hye Kim

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ABSTARCT

Mastoparan B (MP-B) is a cationic tetradecapeptide isolated from the venom of the Taiwan hornet *Vespa basalis*. MP-B exhibits a variety of biofunctional effects such as cardiovascular effect, local edema, antibacterial activity. In this study, we examined the anti-obesity effect of Mastoparan B and its mechanism of action in 3T3-L1 preadipocyte cells. We measured MP-B inhibited adipocyte differentiation by oil red O staining. MP-B decreased the expression of the key adipogenic transcription factors such as CCAAT/enhancer binding protein-alpha (C/EBPα), nuclear receptor peroxisome proliferator-activated receptor gamma (PPARɣ) and sterol regulatory element-binding protein-1 (SREBP-1). These factors are expressed during the late phase of MDI induced differentiation. Moreover MP-B regulated phosphorylation of Akt, glycogen synthase kinase-3 beta (GSK-3β) which play role in adipocyte

differentiation in which insulin and certain growth factors stimulate adipogenesis. The results indicate that MP-B inhibits adipocyte differentiation and accumulation of lipid droplets in 3T3-L1 cell and may have effect for the treatment of obesity.

INTRODUCTION

Adipose tissue plays a crucial role for energy storage, including lipid homeostasis and fatty acid release, but an imbalance between energy intake and consumption can cause obesity, which has become an epidemic in developed countries and developing countries. The rates of obesity and overweight have been to increase in adult, and that the problem has been worsening by penetrating into the child. Obesity can be defined as increased fat mass due to increases in the number and size of adipocytes, which leads to metabolic complications, including type-Ⅱ diabetes, insulin resistance, hyperlipidemia, hypertension, coronary heart disease and cancer [3,10].

To reduce obesity, various programs include lifestyle change, such as exercise and diet, behavioral therapy, and pharmacological treatment [12]. It is still of demand to develop more efficacious and safer anti-obesity products/drugs [10]. Several drugs have been used in the pharmacological treatment of obesity such as orlistat, sibutramine, dinitrophenol, and thyroid hormone. These drugs have been documented to exhibit certain serious side effect, including dry mouth, insomnia, anorexia and valvular heart disease [12]. Thus, new research into healthy foods or drugs without negative side effects is required for the prevention and therapy for obesity [9]. Many studies have demonstrated that natural compounds, such as quercetin, genistein, and esculetin inhibit

adipogenesis [3].

A mouse preadipocyte cell line, 3T3-L1, has been commonly used as an *in vitro* model system to investigate the molecular mechanism of adipogenesis [6]. 3T3-L1 preadipocytes differentiate upon exposure to inducers such as insulin, 3-isobutyl-1-methylxanthine (IBMX), and dexamethasone (DEX) [13]. This treatment initiates early events in adipogenesis, what depends the coordinated regulation of gene expression [9,16].

During adipocyte differentiation, adipogenic transcription factors, such as CCAAT/enhancer-binding protein α (C/EBPα), peroxisome proliferator-activated receptor ɣ (PPARɣ) and sterol regulatory element-binding protein-1 (SREBP-1) are considered to be key regulators of adipogenesis, including adipocyte-specific gene activation in adipocytes [11]. These master factors cooperate to promote the expression of adipocypogenic genes, leading to the terminal differentiation of adipocyte [16]. Moreover, the Akt signaling pathway is important in transducing the proadipogenic effects of insulin and promotes adipocyte differentiation through increase of PPARɣ expression [6].

Mastoparan, the mast-cell degranulating peptide of the vespid venoms, is the major peptide component in many species of hornets [4]. Mastoparan B (MP-B) is a cationic tetradecapeptide (LKLKSIVSWAKKVL-NH_2) isolated from the venom of the Taiwan hornet *Vespa basalis* [19]. MP-B peptide exhibits a wide-range of

biofunctional effects such as stimulation of histamine release from mast cells, erythrocyte lysis, and antimicrobial activity against bacteria [20]. However, its antiobesity effect has not been reported yet. Hence, it remains unknown how Mastoparan B peptide promotes an anti obesity effect in 3T3-L1 cells and high fat diet. Mastoparnan B-12 (LKLKSIVSWAKAVS-NH_2) is a derivative of Matoparan B and was substituted 12^{th} residue by Ala. In the study, we evaluated the inhibitory effect of the Mastoparan B peptide and investigated how it acts to reduce differentiation to adipocytes in 3T3-L1 mouse fibroblasts.

MATERIALS AND METHODS

Peptide synthesis

Following identification by sequencing, the peptide was synthesized by the solid-phase synthesis method on an ASP48S Pepsynthesizer using 9-fluorenylmethoxycarbonyl (Fmoc)-polypeptide active ester chemistry in Peptron Inc, Korea

Cell culture and adipocyte differentiation assay

3T3-L1 mouse preadipocyte cells were purchased from the American Type Culture Collection (Mamassas, VA, USA). 3T3-L1 cells were cultured in Dulbecco's modified eagle's medium (Cellgro, Mediatech, Inc., Manassas, VA, USA) containing 10% fetal calf serum (Gibco®, Grand Island, NY, USA) and 1% penicillin/streptomycin (PAA Laboratories GmbH, Pasching, Austria). Medium was changed every 2-3 days. The cells were incubated at 37℃ in a humidified atmosphere and 5% CO_2 . To induce differentiation, 3T3-L1 cells were cultured in growth media to full confluence. Two days after confluence (day 0), cells were induced to differentiate by changing the medium to DMEM containing 10 μg/ml insulin, 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone (Sigma-Aldrich, Tokyo, Japan) and 10% fetal bovine serum (FBS). After 3 days (day 3), this medium was replaced with 10 μg/ml insulin in DMEM supplemented with 10% FBS. After 2 additional days of

incubation (day 5), this medium was changed to DMEM containing 10% FBS and 2.5 μg/ml insulin. Two days later (day 7), culture medium was replaced with DMEM containing only 10% FBS and incubated for another 2 days.

Cell viability assay

To determine the cytotoxicity and effect on cell viability, 3T3-L1 preadipocyte cells (1×10^4 cells/well) were maintained in 96-well culture plate. After 1 day, cell were treated with sample and incubated for 9 days. After the treatment, 10 μl of WST-1® was added to each well and the plate was incubated at 37℃ for 3 h. The absorbance was mesured at 460 nm with ELISA reader (Molecular Devices, Sunnyvale, CA, USA).

Oil red O staining and Cell quantification

On day 9 after induction of differentiation, cells were stained with Oil-red O. For this assay, cells were washed gently with phosphate-buffered saline (PBS) and fixed in 10% formalin (Junsei Chemical Co., Ltd, Tokyo, Japan) for 1 h. And then cells were stained with filtered Oil-red O solution (60% isopropanol and 40% water) (Sigma-Aldrich, Tokyo, Japan) for 30 min. After staining, the Oil-red O staining solution was removed, and the plate were rinsed three times with water and dried. The stained lipid droplets were viewed on an

inverted microscope (Magnificatiln, ×200). To determine cell quantification, the stained lipid droplets were dissolved in 100% isopropanol (to extract intracellular Oil red O stain) and the absorbance was measured at 520nm.

Measurement of triglyceride assay

The intracellular triglyceride contents were measured in 12-well plates by a commercially available triglyceride quantification kit (BioVision, Inc., San Francisco, CA, USA) according to the manufacturer's instructions. On day 9, the treatment medium was removed and cells were rinsed with PBS. Each well was added with another 200μl PBS and homogenized by sonication for 1 min. The total triglycerides were measured using the assay kit.

Protein extraction and Western blot analysis

3T3-L1 preadipocyte cells were treated with Mastoparan B and MPB-12 during adipocyte differentiation. The treated cells were washed with ice-cold 1× phosphate-buffered saline (PBS) and collected in lysis buffer [(50 mM Tris-C1 (pH 7.5), 150 mM NaCl, 1 mM DTT, 0.5% NP-40, 1% Triton X-100, 1% Deoxycholate, 0.1% SDS and cocktail of proteinase inhibitors (PMSF, EDTA, aprotinin, leupeptin, prostatin A; Intron biotechnology, Gyeonggi, Korea)] on ice. After incubation on ice for 30 min, the insoluble materials were removed by centrifugation at

14,000 rpm for 20 min. The protein contents of the cell lysates were determined by a Protein Quantification Kit (CBB solution®) (Dojindo Morecular Technologies, Rockvile, MD, USA) with bovine serum albumin (BSA) as standard. The absorbance was measured at 595nm. An aliquot from each sample was boiled for 5 min and then resolved by 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were electrotransferred to a nitrocellulose membrane (PALL Life Sciences, Pensacola, MI, USA) and blocked in PBST buffer (135 μM NaCl, 2.7 mM KCl, 4.3 mM $NaPO_4$, 1.4 mM KH_2 PO_4 and 0.5% Tween-20) containing 5% skim milk. After blocking, the membrane was probed with primary antibodies (anti-C/EBPα, anti-PPARɣ, anti-SREBP1, anti-mTOR, anti-phosphor-mTOR (ser2481), anti-GSK3β, anti-phosphor-GSK3β (ser9), anti-Akt, anti-phosphor-Akt (ser473), phosphor-p44/42MAPK (ERK1/2) (Thr202/Try204) (197G2), phosphor-AMPKα1/2 (Thr172), anti-β-actin; Cell Signaling Technology Inc., Beverly, MA, USA) and washed three times with PBST buffer followed by incubation for 1 h with horseradish peroxodase-conjugated anri-rabbit IgG or anti-mouse IgG as second antibodies (Cell Signaling Technology Inc.). The blots were then washed with PBST buffer and were visualized by an enhanced chemiluminescent (ECL) detection solution (Pierce, Rockford, IL, USA).

Statistical analysis

All statistical analyses were performed using the software Prism 5.0

software (GraphPad, San Diego, CA, USA) for windows. It was used to determine the statistical significance of differences between values for various experimental and control group. Determinations were performed in triplicate and the results are presented as means±SD error of the mean (S.E.M.). ANOVA post hoc test and subsequently Dunnett's multiple tests were used for statistical analysis.

RESULTS

Peptide residues substitute for Ala

Nine peptides used in this study, the primary structure of MP-B and its analogs were listed in Table 1. Mastoparan B and its derivatives were synthesized by using Fmoc-solid phase synthesis. Each derivative's residue was replaced by Ala.

Peptide	Sequence
MPB	**L-K-L-K-S-I-V-S-W-A-K-K-V-L-NH2**
MPB-1	**A**-K-L-K-S-I-V-S-W-A-K-K-V-L-NH2
MPB-3	L-K-**A**-K-S-I-V-S-W-A-K-K-V-L-NH2
MPB-6	L-K-L-K-S-**A**-V-S-W-A-K-K-V-L-NH2
MPB-7	L-K-L-K-S-I-**A**-S-W-A-K-K-V-L-NH2
MPB-11	L-K-L-K-S-I-V-S-W-A-**A**-K-V-L-NH2
MPB-12	L-K-L-K-S-I-V-S-W-A-K-**A**-V-L-NH2
MPB-13	L-K-L-K-S-I-V-S-W-A-K-K-**A**-L-NH2
MPB-14	L-K-L-K-S-I-V-S-W-A-K-K-V-**L**-NH2

Table 1. Primary structure of MP-B and its analogs

Comparative effects of Mastoparan B peptides in Inhibition of Adipogenesis in 3T3-L1 Adipocytes

Microscopic analysis of Oil-red-O stained differentiating 3T3-L1 adipocytes, cultured in the absence and presence of MP-B and its derivatives, revealed the accrual of intracellular lipid droplets. Quantification of the extracted Oil-red-O stain showed that the lipid content was reduced by Mastoparan B and Mastoparan B-12 when treated at 10μM as compared to the adipogenesis control. As a result, MP-B and MP-B 12 were used in this study.

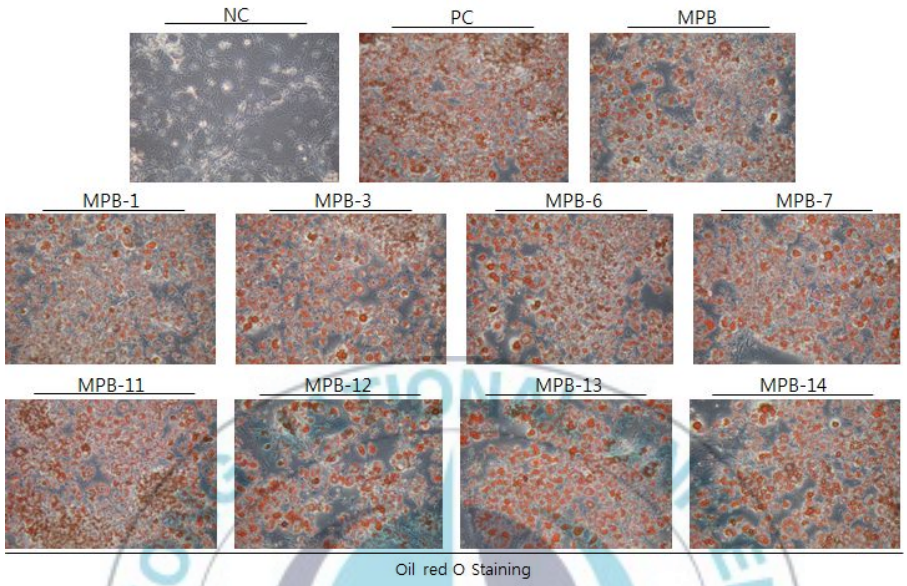
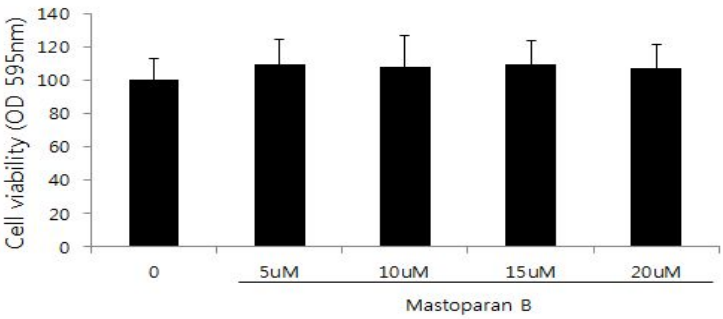


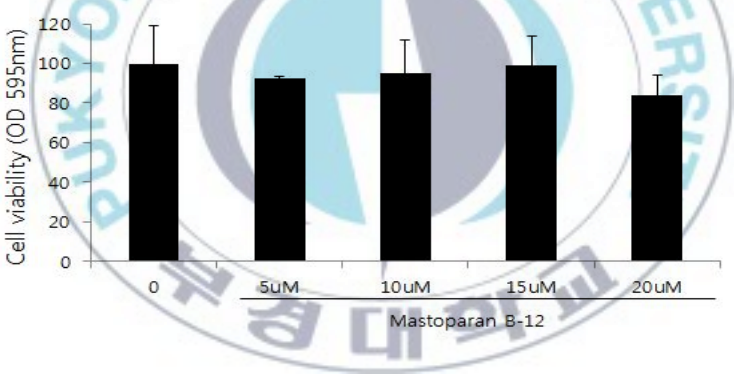
Figure 1. Intracellular lipids were stained with Oil red O. 3T3-L1 cells were treated with MP-B peptides at 10μM.

Effect of Mastoparan B on 3T3-L1 cells viability

This study performed a WST-1 assay to analyze the viability of 3T3-L1 preadipocyte cells treated with Mastoparan B 10μM, Mastoparan B-12 10μM and 15μM for 9 days. In results, MP-B and MP-B-12 did not show any effect on cell viability and cytotoxicity. Therefore, the concentration of MP-B and MP-B-12 was chosen for further experiments.



(A)

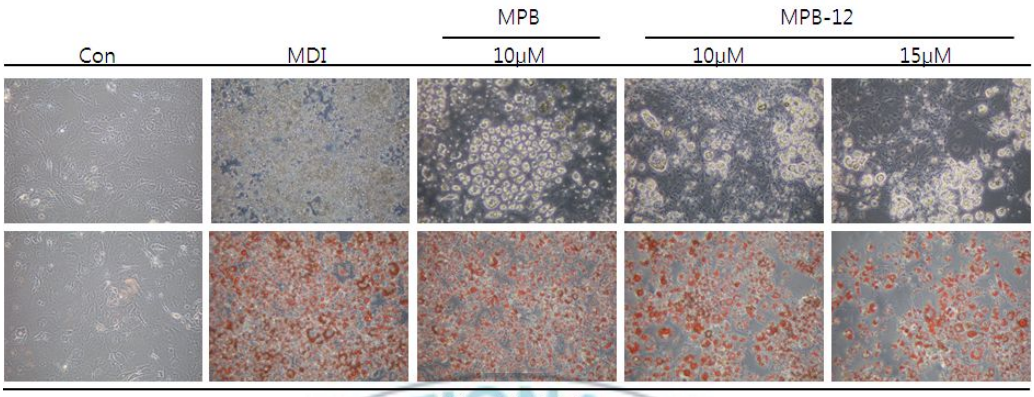


(B)

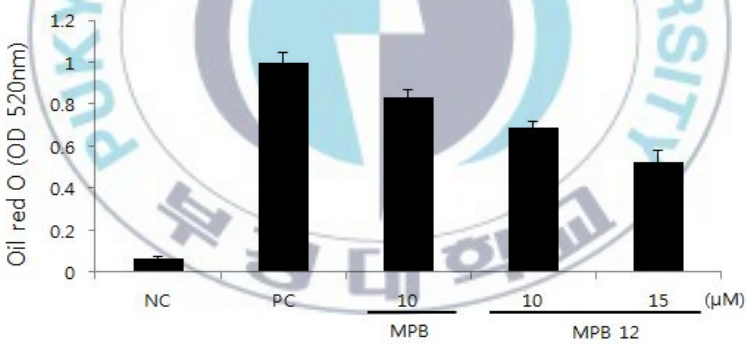
Figure 2. Effect of Mastoparan B and Mastoparan B 12 on the ceell viability. 3T3-L1 cells were treated with Mastoparan B and Mastoparan B -12 for 9 days. Cell viability was determined by WST 1 assay.

Mastoparan B inhibits differentiation of 3T3-L1 preadipocytes

To investigate the effect of Mastoparan B on adipocyte differentiation, 3T3-L1 cells were induced to differentiation with MDI (0.5 mM 3-isobutyl-1-methylxanthine, 0.5 μM dexamethasone and 1 μg/ml insulin) in the presence or absence of Mastoparan B for 9 days. The cells were treated with MPB 10 μM, MPB-12 10 and 15μM. After differentiation, the effect of Mastoparan B on the lipid accumulation of adipocytes was measured by Oil Red O staining. The differentiation of preadipocytes into adipocytes is associated with an increased number of Oil Red O stained cells due to lipid accumulation. Fig. 2A shows the result of Oil Red O staining under an inverted microscope. Both Mastoparan B and Mastoparan B 12 inhibited the differentiation of 3T3-L1 cells. And inhibition of Mastoparan B 12 on lipid accumulation of adipocytes was better compared with Mastoparan B. Also, Mastoparan B 12 inhibited the differentiation of 3T3-L1 preadipocytes in a dose-dependent manner. Treatment with Mastoparan B peptides significantly decreased lipid droplets compared with MDI-treated cells. These results demonstrated that Mastoparan peptides inhibited the differentiation of preadipocytes.



(A)



(B)

Figure 3. The effects of Mastoparan B and Mastoparan B 12 on lipid accumulation of 3T3-L1 adipocytes. (A) The intracellular lipid accumulation stained by Oil Red O and observed by an inverted

microscope. (B) The absorbance of Oil Red O dissolved in isopropanol was determined at 520 nm. Con, 3T3-L1 preadipocytes; MDI, fully differentiated adipocytes; MPB 10μM, fully differentiated adipocytes (MDI + 10μM MPB); MPB-12 10μM, fully differentiated adipocytes (MDI + 10μM MPB-12); MPB-12 15μM, fully differentiated adipocytes (MDI + 10μM MPB-12).

Mastoparan B peptides suppressed triglycerides synthesis

To measure the level of intracellular triglycerides, 3T3-L1 preadipocytes were differentiated with Mastoparan B peptides for 9 days. Triglycerides play an important role as transporters of fatty acids as well as serving as an energy source and were used as a major marker of adipogenesis. In this result, lipid accumulation was quantified on day 9 of the differentiation period and showed that treatment of Mastoparan B peptides reduce triglyceride content and the inhibition of intracellular triglyceride accumulation in 3T3-L1 cells. Both MP-B and MP-B12 reduce triglyceride content but reduction of lipid accumulation by MP-B12 were greater than MP-B. The inhibitory effect of MP-B12 in triglyceride accumulation during adipogenesis were also dose-dependent manner.

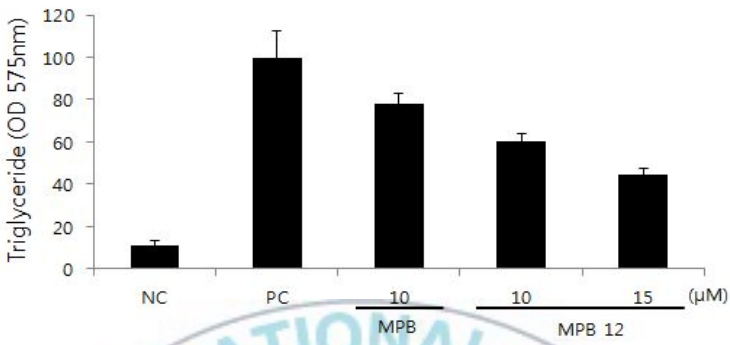


Figure 4. Mastoparan B peptides-mediated suppression of the intracellular triglyceride level in 3T3-L1 cells.

Mastoparan B inhibits the expression of adipogenic transcription factors

Next, to examine whether Mastoparan B peptides inhibit adipocyte differentiation through the down-regulation of adipogenic transcription factors by western blot analysis. Preadipocytes were induced to differentiate in induction medium with Mastoparan B peptides and harvested for 9 days. Differentiation of preadipocyte into adipocyte is tightly regulated by a sequential activation of several transcriptional factors, including C/EBPα, PPARγ and SREBP-1. They are necessary for the expression of adipocyte-specific genes. As shown in Fig. 3, the protein expression levels reduced by Mastoparan B peptides. Particularly, PPARγ and C/EBRα are master adipogenic transcription facotors. In result, the protein expressions of PPARγ and C/EBRα were more decreased by Mastoparan B-12. And Mastoparan B 12 inhibited the protein expression in a dose-dependent manner. These results demonstrate that Mastoparan B peptides inhibit adipogenesis through the down-regulation of adipogenic transcription factors and inhibition effect of Mastoparan B-12 is better compare with Mastoparan B.

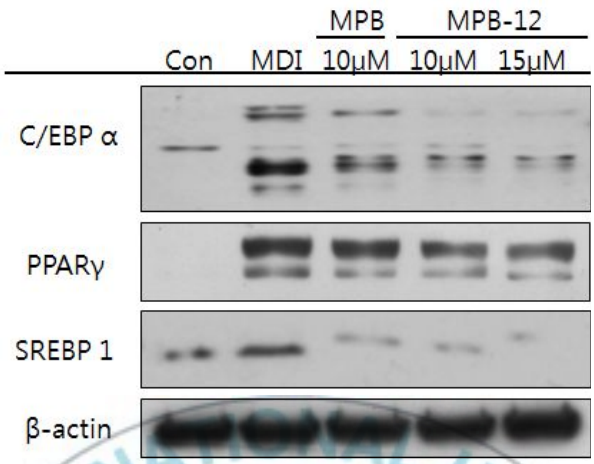


Figure 5. Effect of Mastoparan B peptides on adipogenic transcription factor protein levels. Western blot analysis was performed with antibodies against C/EBPα, PPARγ, SREBP-1 and β-actin.

Adipogenesis inhibition through the suppression of ERK1/2 and AMPKα phosphorylation

Many studies have suggested that MAPKs promote early-stage adipocyte differentiation by activating transcription factors. The activation of extracellular signal regulated kinase 1/2 (ERK1/2) would be necessary for adipogenesis [20]. And ERK1/2 regulates expression of AMPK α. The AMP-activated protein kinase (AMPK) functions as a sensor of cellular energy status. AMPK provides an upstream signal of peroxisome proliferator-activated receptor γ (PPARγ) and inhibits differentiation of preadipocytes into adipocytes. In this study, Mastoparan peptides merkedly decreased the phosphorylation of ERK1/2 and AMPKα. Moreover, Mastoparan B - 12 inhibits expression of ERK1/2 and AMPK-α phosphorylation in a dose-dependent manner more than Mastoparan B.

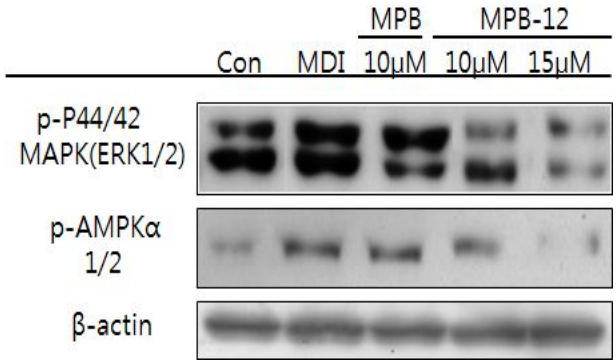


Figure 6. Phosphorylation of ERK1/2 and AMPKα is decreased by Mastoparan B peptides.

The phosphorylation of Akt pathway in differentiation of 3T3-L1 cells

One of the established signaling mechanisms for increasing PPARɣ gene expression is mediated by Akt in adipocyte differentiation. Akt is omportant in glucose regulation and lipid metabolism in insulin signaling, and GSK3β is a downstream target of Akt in adipocyte differentiation. In this study, the effect of Mastoparan B peptides on the levels of phosphorylated Akt during adipocyte differentiation of 3T3-L1 cells. Treated cells by Mastoparan B pepties effectively suppressed MDI-induced phosphorylation of Akt and its upstream signals, such as PI3K p110 isoforms (α,β,ɣ) and mTOR. The PI3K signaling pathway plays an important role in regulating adipo mass, obesity, and diabetes. PI3 K is a signal transduction system at the downstream of an insulin receptor (IR) [23]. Glycogen synthesis kinase 3 beta (GSK3β) is a critical downstream signaling protein of the phosphoinositide 3-kinase (PI3K)/Akt pathway. Insulin signaling activates Akt through PI3K and induces serine/threonine phosphorylation of downstream target GSK3β [25]. The phosphorylation of insulin stimulated Akt was reduced after treatment with Mastoparan B peptides and Mastoparan B-12 decreased the level of phosphor-Akt in a dose-dependent manner. In addition, insulin-stimulated phosphorylated GSK3β decreased with the addition of Mastoparan B peptides. These results demonstrate the MP-B peptides

inhibits the phosphorylation of Aktm which suppresses the phosphorylation of its substrate kinase GSK3β. In case of PI3K p110 isoforms, Mastoparan B suppressed expression compared with treated Mastoparan B-12. Mastoparan B specifically inhibits expression of PI3K and Mastoparan B-12 reduced cellular lipid accumulation in a dose-dependent manner in 3T3-L1 cells.

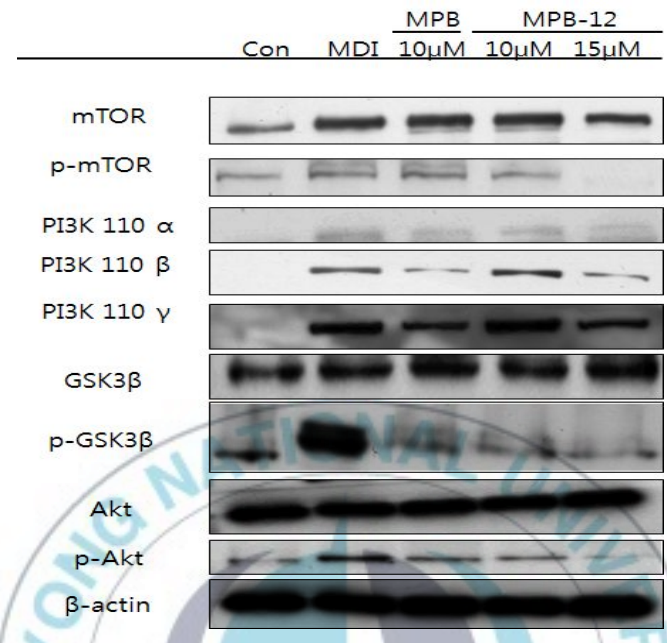


Figure 7. Effect of Mastoparan B peptides on phosphorylation of Akt, GSK3β and PI3K p110 isoforms in 3T3-L1 adipocytes.

DISCUSSION

In the present study, we evaluated the effect of Mastoparna B peptides on adipocyte differentiation as well as its inhibitory mechanisms on adipogenesis in 3T3-L1 cells and anti-obesity. Mastoparan B peptides have antiadipogenic effect through inhibition of C/EBPα, PPARγ, and SREBP-1 expression and phosphorylation of ERK1/2, AMPKα and Akt signaling pathway in 3T3-L1 cells. Treated with Mastoparan B peptides cells attenuated lipid accumulation as determined by Oil-red O staining and a triglyceride accumulation assay without cytotoxicity.

Adipocyte differentiation and fat accumulation are associated with the occurrence and development of obesity. A reduction of adiposity is related to the inhibition of angiogenesis along with reduction of adipocyte numbers and the lipid content of adipocytes. The differentiation of preadipocytes into adipocytes is regulated by a complex network of transcription factors [25]. The main transcription factors are nuclrear receptor PPARγ, C/EBPα and SREBP-1, which are important for the process of adipocyte differentiation. PPARγ and C/EBPα are important of the entire terminal differentiation process [22]. Moreover, PPRRγ is activated by fatty acid, and fat accumulation is associated with PPARγ activation. PPARγ and C/EBPα activate the expression of genes involved in adipogenesis, such as aP2, FAS and LPL, to trigger the synthesis of fatty acids and triglycerides [25]. And the SREBP transcription factors

regulate genes related to the metabolism of lipids and cholesterol [16]. SREBP-1 regulates lipogenic gene expression associated with fatty acid synthesis, which leads to increased synthesis of TG, and can contribute to the expression of PPARγ ligands [11]. Mastoparan B peptides reduced expression of transcriptional factors compared with only MDI cells, Mastoparan B-12 suppressed adipogenesis in 3T3-L1 cells more than Mastoparan B (Figure 5).

The ERK1/2 pathway is upstream of PPARγ and C/EBPα. Activating of PPARγ and C/EBPα was promoted by ERK activation in 3T3-L1 preadipocyte differentiation [7]. This study observed that the Mastoparan B peptides inhibited the phosphorylation of ERK1/2 in adipogenesis (Figure 6). And AMPK is a key sensor of fuel and energy status in skeletal muscle, involving in glycogen breakdown, glycolysis, glucose uptake and fatty acid oxidation, together with many changes in gene expression [23]. AMPK phosphorylation increased during the early phase of differentiation, these result suggest the Mastoparan B peptides inhibits adipogenesis via AMPK signaling during the early phase of MDI-induced differentiation (Figure 6).

The serine/threonine kinase Akt is particularly important in mediating adipocyte differentiation and the metabolic actions of insulin. Akt phosphorylates and regulates a large number of substrates involved in a diverse array of biological processes. GSK3β is a critical downstream signaling protein for the phosphoinositide 3-kinase (PI3K)/Akt pathway

[25]. This study showed that the serine phorphorylation of Akt was decreased by Mastoparan B peptides and subsequently attenuated the levels of phosphorylation GSK3β. These result indicated that inhibiting Akt phosphorylation reduced the phosphorylation of downstream signaling components (Figure 7).

In the present study, results show that Mastoparan B peptides suppresse adipogenesis in 3T3-L1 cells by downregulating the expression of PPARɣ, C/EBPα and SREBP-1 through the phosphorylation of ERK1/2, AMPK, Akt signaling pathway. Therefore, these data indicate that Mastoparan B pepetides are potent and Mastoparan B-12 has inhibition activity of adipocyte more than Mastoparan B. Futher studies are needed to elucidate the potential role of kinase inhibitors.

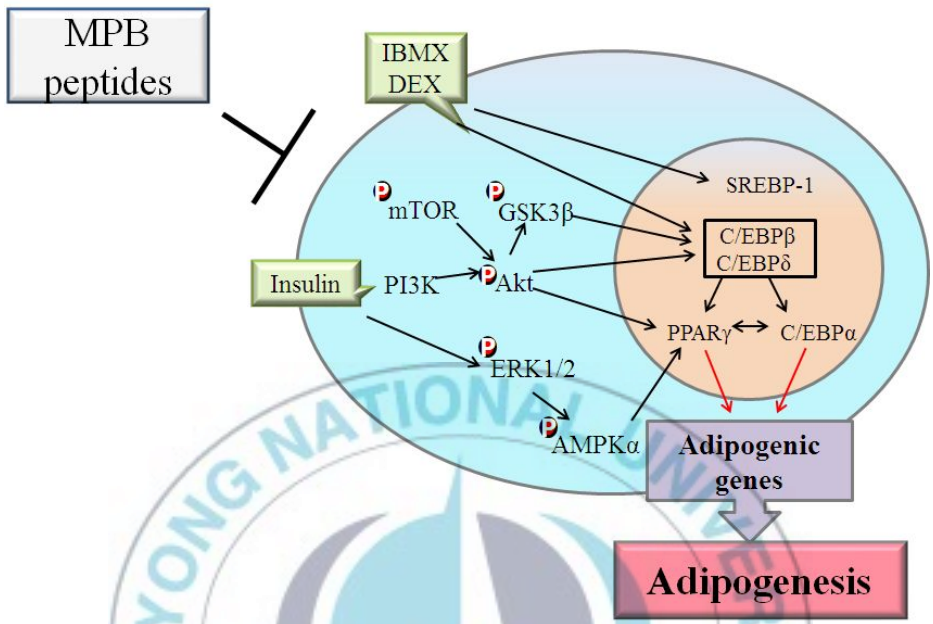


Figure 8. Predictable mechanism of Mastoparan B on the inhibition of 3T3-L1 adipocytes differentiation.

국문초록

Mastoparan B는 타이완 말벌인 *Vespa basalis*의 독에서 분리된 cationic tetradecapeptide로 심혈관 효과, 국소 부종, 항균 활성과 같은 다양한 생물 활성을 가진다고 알려져 있다. 본 연구는 Mastoparan B의 항비만 효과와 3T3-L1 전지방세포에서 억제 기작을 확인하는 실험을 진행하였다. Mastoparan B의 세포 내 지방축적에 대한 효과는 Oil red O 염색을 통해 확인하였다. 지방분화 관련 단백질의 발현을 확인하기 위해 western blot으로 분석한 결과 지방산합성 및 분화를 유도하는 주요 유전자발현을 증가시키는 것으로 알려진 전사인자 C/EBPα, PPARɣ, SREBP-1이 Mastoparan B 펩타이드에 의해 발현이 감소됨을 확인 하였으며, 이는 지방분화 억제에 효과가 있음을 알 수 있다. 그리고 지방분화 연구에 많이 알려진 PI3K/Akt pathway의 발현이 감소되었고, 이에 의해 조절되는 GSK-3β의 발현 또한 줄어드는 것을 확인 하였다. Adipogenesis가 이뤄지면서 phosphorylation이 일어나게 되고 이 관련 AMPK, ERK의 발현도 억제되었다. 이로써 Mastoparan B 펩타이드가 3T3-L1 전지방세포에서 지방세포로 분화하는 과정에서 지방세포의 생성 및 축적을 억제하고 이와 관련된 전사인자 및 phosphorylation 된 단백질 발현을 감소시키므로 지질대사 개선에 효과가 있다고 생각된다.

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2년이라는 시간이 너무나 짧게 지나가버렸습니다. 먼저 여러 가지로 부족하고 모든 것이 낯설었던 저에게 많은 애정과 믿음으로 이 자리까지 이끌어 주신 김군도 교수님께 깊은 감사의 말씀을 드립니다. 그리고 바쁘신 와중에도 이 논문을 완정할 수 있도록 따뜻한 격려와 아낌없는 조언을 해주신 송영환 교수님, 최태진 교수님께 감사드립니다. 기초가 부족한 저에게 많은 가르침을 주신 이명숙 교수님, 언제나 학과를 걱정하고 좋은 말씀을 해주신 김영태 교수님, 김경호 교수님, 전용재 교수님께 감사드립니다. 또 소중한 물질로 논문에 많은 도움을 주시고 항상 제 걱정을 해주시는 멋진 박남규 교수님 및 고혜진 박사님을 비롯한 연구원분들께도 감사의 마음을 전합니다.

2년 동안 제게 과분한 애정과 평생 잊지 못할 추억을 선물해준 세포신호전달 연구실 선후배들에게 감사의 말을 전하고 싶습니다. 항상 격려를 아끼지 않으셨던 성영애 박사님, 지금은 호주에서 멋진 연구를 하고 있을 진수선배, 언제나 똑똑한 주당 우석선배, 아기자기하고 꼼꼼한 덕현이, 늘 착하고 잘 먹어서 예쁜 난희, 새로운 곳에서 박사과정을 시작한 성석선배, 나의 부름에 언제나 응답하는 종용이, 누나 걱정해주는 의젓한 민석이, 열심히 실험하는 창원이, 대학원 들어와서 열심히 실험할 지현이, 막내가 들어와도 항상 막내 민재, 아침 잠꾸러기 기숙사 주민 형욱이, 나이보다 성숙한 멘탈 전일이, 일미 수업 때 많이 도와준 기일이, 실험실 신입생 경록이, 떨어져 있지만 졸업동기인 Coy 너무 고생 많았고, 늘 고마워. 그리고 만남부터 모든 것을 공유하고 지금까지도 변함없이 서로 비밀이 없는 사이 쪼미! 네가 있어서 그 시간동안 더 많이 배우고 쉽게 적응 할 수 있었던 것 같아서 많이 고마워. 또한, 연구실 생활을 통해 즐거운 인연을 맺은 항상 밝은 응급실 초원이, 실험을 통해 훨씬 가까워지고 많은 도움을 준 순진이, 더 친해지지 못한 게 아쉬운 착한남자 은수선배, 바쁜 와중에도 잘 챙겨준 보근오빠 및 학과생활에 있어 많은 도움을 준 미진이와 많은 애정을 주신 다른 졸업동기, 선배, 후배님들에게도 감사를 전하며 앞으로 모두에게 좋은 일들만 가득하시길 바랍니다.

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