Original Article

Melittin Inhibits DU-145 Human Refractory Prostate Cancer Cell Growth Through Induction of Apoptosis Via Inactivation of NF-κB

Choi Chul-hoon and Song Ho-sueb

Dept. of Acupuncture & Moxibustion, College of Oriental Medicine, Kyungwon University

국문초록

Melittin이 NF-κB의 불활성화를 통한 DU-145 전립선 암세포의 성장 및 세포자멸사 유도에 미치는 영향

최철훈 · 송호섭

경원대학교 한의과대학 침구학교실

목적 : 이 연구는 봉약침의 주요성분인 멜리틴이 NF-κB의 활성억제를 통하여 세포자멸사를 유도하고, 전립선 암세포주인 DU-145 세포의 성장을 억제하는지를 확인하고 멜리틴의 NF-κB 활성억제기전을 살펴보고자 하였다.

방법 : 멜리틴을 처리한 후 DU-145의 성장억제를 관찰하기 위해 WST-1 assay를 시행하였고, 세포자멸사의 관찰에는 DAPI staining assay를 통한 세포형태관찰을 시행하였으며, 염증관련유전자 발현 관찰에는 western blot analysis를 시행하였고, 세포자멸사와 연관된 NF-κB의 활성 변화를 관찰하기 위해 EMSA와 luciferase assay를 시행하였으며, DU-145에서 멜리틴과 NF-κB의 상호작용을 관찰하기 위해 transient transfection assay를 시행 시 세포생존율과 NF-κB의 활성 변동을 측정하였다.

결과 : DU-145 세포에 멜리틴을 처리한 후, 전립선암세포의 성장, 세포자멸사의 유발, 염증관련유전자 발현 및 NF-κB의 활성, NF-κB의 p50 치환 후 NF-κB의 활성과 DU-145 세포 증식에 미치는 영향을 관찰하여 다음과 같은 결과를 얻었다.

1. DU-145 세포에서 멜리틴을 처리한 후 세포자멸사가 유도되어 세포성장이 억제되었다.
2. DU-145 세포에서 멜리틴을 처리한 후 염증관련유전자 발현 및 NF-κB의 활성에 유의한 감소를 나타내었다.
3. DU-145 세포에서 NF-κB의 p50와 IKK들을 치환하여 작용기를 없애고 멜리틴을 처리하였을 경우에도 세포활성 및 NF-κB의 활성의 유의한 감소를 나타내었다.

· Acceptance : 2009. 5. 18. · Adjustment : 2009. 6. 4. · Adoption : 2009. 6. 4.
· Corresponding author : Song Ho-sueb, Department of Acupuncture & Moxibustion, Dongincheon Gil Medical Center, 117 Yong-dong Jung-gu Incheon, Republic of Korea
Tel. 82-32-770-1212 E-mail : hssong70@kyungwon.ac.kr

결론 : 이상의 결과는 멜리틴이 NF-κB의 활성 억제를 통하여 인간 전립선암 세포주인 DU-145의 세포자멸사를 유발함으로써 증식억제 효과가 있음을 입증한 것으로 전립선암의 예방과 치료에 대한 효과적인 치료제 개발에 도움이 될 것으로 기대된다. 다만 그 기전에서 멜리틴은 기존연구와 달리 NF-κB p50 및 IKK들의 작용기와 직접적으로 상호작용을 하지는 않는 것으로 보이므로 심화 연구를 요한다.

핵심 단어 : 봉독, 멜리틴, 전립선암, DU-145, 세포자멸사, NF-κB, transfection, mutant p50 and IKKs

Ⅰ. Introduction

Prostate cancer is the most common cancer next to skin cancer and the second leading cause of cancer death among men in the United States[1]. Initially it is characterized by androgen-dependent growth and androgen deprivation therapy(ADT) remains the most important therapy for advanced Prostate Cancer[1-4]. However, as the disease progresses, ADT eventually fails leading to the development of androgen-independent prostate cancer[2].

One of the goals of current researches have been the identification of new agents for the prevention or treatment of prostate cancer[5], and moreover, elucidating important molecular mechanisms of specific toxin-receptor and/or ion channel complexes have been largely studied in drug discovery using natural toxins[6-8]. Melittin is known as a major component of bee venom toxin, interacting with NF-κB signal molecules by means of protein-protein bindings with the cysteine residues in NF-κB signal molecules[9]. Increasing studies have demonstrated that melittin inhibits cancer growth, and induction of apoptotic cell death[10]. Park et al. reported that melittin inhibited human prostate cancer cell growth through induction of apoptotic cell death via down regulation of NF-κB and alteration of expression of apoptosis regulatory proteins[11]. Lee et al.[12], in their NF-κB inactivation related mechanism study, elucidated that MT or BV inhibited cell viability and NF-κB activity dose dependently in the LNCaP cells transfected with p50(C62S) mutant as well as in wild LNCaP cells, implying the existence of another target except for cysteine residue with sulf hydryl groups in molecules of NF-κB signal pathway including p50, IKKs, inconsistent with previous reports[9,11,13].

In the present study, I therefor conducted a mechanism study, determining whether melittin exerts inhibitory effect on human prostate cancer DU-145 cell proliferation via induction of apoptotic cell death through inactivation of NF-κB ,and corroborating whether cysteine residue of IKKs as well as p50 is target of melittin down regulating NF-κB activity.

Ⅱ. Materials and method

1. Materials

Melittin(MT) was purchased from Sigma Chemical Co.(St. Louis, MO, USA). goat polyclonal antibody to COX-2(1 : 500), and TNF(1 : 500), mouse polyclonal antibody to iNOS(1:500) and $cPLA_2$(1 : 500) used in Western blot analysis were purchased from Santa Cruz Biotechnology(Santa Cruz, CA). T4 polynucleotide kinase was obtained from Promega (Madison, WI). Poly(dI-dC), horseradish peroxidase-labeled donkey anti-rabbit secondary antibody, and ECL detection reagent were obtained from Amersham Pharmacia Biotech(Piscataway, NJ). Reagents for sodium dodecyl sulfate(SDS) - polyacrylamide gel electrophoresis were purchased from Bio-Rad (Hercules, CA). All other reagents were purchased

from Sigma unless otherwise stated.

2. Cell culture

The DU-145 human prostate cancer cell was obtained from ATCC(American Type Culture Collection, Rockville, MD). Prostate cells were cultured in RPMI-1640 medium(Life Technologies Inc., Gaithersberg, MD) supplement with 10% fetal calf serum(FCS; Collaborative Biomedical Products, Bedford, MA) and antibiotics, penicillin/streptomycin (100unit/ml, Bioproducts, Walkersville, MD) Cell cultures were then maintained at 37℃ in a humidified atmosphere of 5% CO_2

3. Cell viability assay(WST-1 assay)

Cells were plated at a density of 1 × 105 cells per well in 96-well plate and then subconfluent cells were exposed to different doses of melittin(0.5-2.5㎍) or bee venom(1-10㎍) for 12, 24, 48, 72hr. After treatment, cell viability was measured by WST-1 assay(Dojin Laboratory, Kumamoto, Japan) according to the manufacturer's instructions. WST-1 solution was added to cells in 96-well plates, cells were incubated at 37.5℃ for 1hr, and the optical density of each well was read at 450nm.

4. Morphologic evaluation of apoptosis

Apoptosis assays were performed using the 4,6-diamidino-2-phenylindole(DAPI) staining. DU-145 cells were cultured in the absence or presence of increasing concentrations of melittin, and apoptosis induction were evaluated after 24hr. Apoptotic cells were determined by the morphological changes after DAPI staining under fluorescence microscopic observation(DAS microscope, 100 or 200x; Leica Microsystems, Inc., Deerfield, IL). For each determination, three separate 100-cell counts were scored. Apoptosis was expressed as a percentage calculated from the number of cells with apoptotic nuclear morphology divided by the total number of cells counted.

5. Western blot analysis

Cells were homogenized with lysis buffer [50mM Tris pH 8.0, 150mM NaCl, 0.02% sodium azide, 0.2% SDS, 1mM PMFS, 10㎕/ml aprotinin, 1% igapel 630(Sigma-Aldrich, St. Louis, MO, USA), 10mM NaF, 0.5mM EDTA, 0.1mM EGTA and 0.5% sodium deoxycholate], and centrifuged at 23,000g for 1hr. Equal amount of proteins(80㎍) were separated on a SDS/12%-polyacrylamide gel, and then transferred to a nitrocellulose membrane (Hybond ECL, Amersham Pharmacia Biotech Inc., Piscataway, NJ). Blots were blocked for 2hr at room temperature with 5%(w/v) non-fat dried milk in Tris-buffered saline[10mM Tris(pH 8.0) and 150mM NaCl] solution containing 0.05% tween- 20. The membrane was incubated for 5hr at room temperature with specific antibodies such as goat polyclonal antibody to COX-2(1 : 500), mouse polyclonal antibody to iNOS(1 : 500) and $cPLA_2$(1 : 500) (Santa Cruz, CA, USA Santa Cruz Biotechnology Inc). The blot was then incubated with the corresponding conjugated anti-rabbit immunoglobulin G-horseradish peroxidase(Santa Cruz Biotechnology Inc). Immunoreactive proteins were detected with the ECL western blotting detection system. The relative density of the proteinbands was scanned by densitometry using MyImage(SLB, Seoul, Korea), and quantified by Labworks 4.0 software(UVP Inc., Upland, California).

6. Preparation of nuclear extracts and electromobility shift assays

It was performed according to the manufacturer's recommendations(Promega, Madison, WI). Briefly, 1 × 10^6 cells/ml was washed twice with 1 × PBS, followed by the addition of 1ml of PBS, and the cells were scraped into a cold Eppendorf tube. Cells were spun down at 15,000g for 1min, and the resulting supernatant was removed. Solution A (50mM HEPES, pH 7.4, 10mM KCl, 1mM EDTA, 1mM EGTA, 1mM dithiothreitol, 0.1㎍/ml phenylmethyl-sulfonyl fluoride, 1㎍/ml pepstatin A, 1㎍/ml

leupeptin, 10μg/ml soybean trypsin inhibitor, 10μg/ml aprotinin, and 0.5% Nonidet P-40) was added to the pellet in a 2 : 1 ratio(v/v) and allowed to incubate on ice for 10min. Solution C(solution A + 10% glycerol and 400mM KCl) was added to the pellet in a 2 : 1 ratio(v/v) and vortexed on ice for 20min. The cells were centrifuged at 15,000g for 7min, and the resulting nuclear extract supernatant was collected in a chilled Eppendorf tube. Consensus oligonucleotides were end-labeled using T4 polynucleotide kinase and [g^{-32}P] ATP for 10min at 37℃. Gel shift reactions were assembled and allowed to incubate at room temperature for 10min followed by the addition of 1μl(50,000-200,000cpm) of ^{32}P-labeled oligonucleotide and another 20min of incubation at room temperature. For supershift assays, nuclear extracts from cells treated with melittin(0.5-2.5μg) were incubated with specific antibodies against the p50, p65 and Rel-A NF-κB isoforms for 1hr before EMSA. For competition assays, nuclear extracts from cells treated with melittin(0.5-2.5μg) were incubated with unlabelled NF-κB oligonuclaotide (50X, 100X and 200X) or labeled SP-1(100X) and AP-1(100X) for 30min before EMSA. Subsequently 1μl of gel loading buffer was added to each reaction and loaded onto a 6% nondenaturing gel and electrophoresed until the dye was three-fourths of the way down the gel. The gel was dried at 80℃ for 1hr and exposed to film overnight at 70℃. The relative density of the DNA-protein binding bands was scanned by densitometry using MyImage (SLB, Seoul, Korea), and quantified by Labworks 4.0 software(UVP Inc, Upland, California).

7. Assay of luciferase activity

DU-145 cells were cotreated with melittin(0.5-2.5μg) and TNF-α. Luciferase activity was measured using a luciferase assay kit(Promega) and WinGlow software, according to the manufacturer's instructions(Berthold, Wildbad, Germany).

8. Transient transfection assay

A fusion gene containing pCMV promoter and p50, IKKs(IKKα, IKKβ) mutant was used. DU-145 cells were transfected with these mutant genes constructs. Cell viability and NF-κB, IKKs activity were then measured after stimulating the cells for 24hr with melittin(0.5-2.5μg) for 24hr.

9. Statistical analysis

Data were analyzed using one-way analysis of variance followed by Tuckey test as a post hoc test. Differences were considered significant at $p< 0.05$.

Ⅲ. Results

1. Inhibition of DU-145 cell growth

Once the cells were exposed to MT(0.5-2.5μg), the cells were not grown, and died in a dose dependent manner. To evaluate an effect of MT on the cell growth of DU-145 cells, I analyzed cell viability using WST-1 assay and direct cell dose dependently in 1 and 2.5μg/㎖ of MT. The

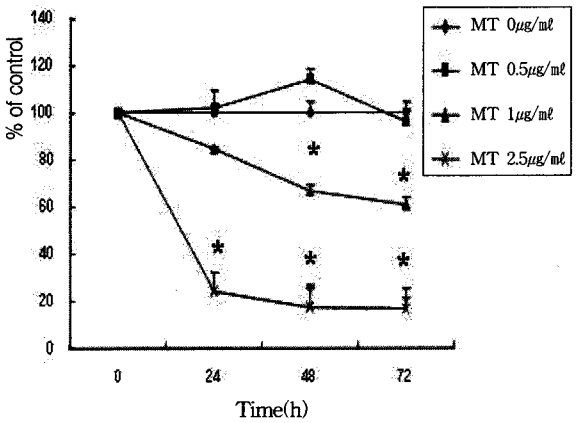

Fig. 1. DU-145 cell viability by MT according to time courses

MT and * represents melittin and $P<0.05$, significance, compared with control respectively.

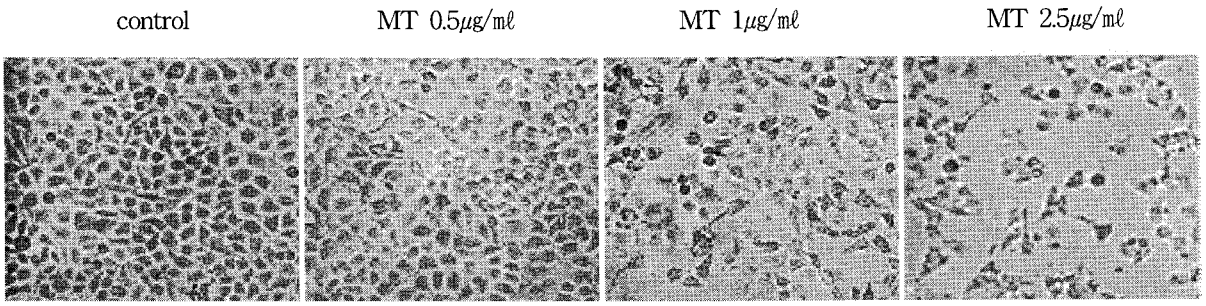

Fig. 2. Morphologic change of DU-145 cells treated by MT

Morphological changes were observed under microscope(magnification, 200×). The figures are representative of three experiments, with triplicate of each experiment. MT represents melittin.

counting. MT inhibited prostate cancer cell growth percentage of control significantly decreased by 1μg/mℓ of MT at 48 and 72hr was 66.5±3.2 and 60.5±3.8%, which of control significantly decreased by 2.5μg/mℓ of MT at 24, 48 and 72hr was 24.0±7.9, 17.4±9.3 and 16.6±4.6%(Fig. 1).

2. Morphologic evaluation of apoptosis

To delineate whether the inhibition of cell growth by MT was due to increase of the induction of apoptosis, I evaluated change of the chromatin morphology of human prostate cancer cells using DAPI staining. Consistent with the loss of viability, DU-145 cell demonstrated the characteristics of apoptotic morphologic change including cell shrinkage with irregular shape, cytoplasmic blebbing, chromatin condensation, DNA fragmentation and apoptotic bodies, etc(Fig. 2).

3. Inhibition of NF-κB

It was demonstrated that MT negatively regulates NF-κB and suppress expression of inflammation related genes including iNOS and COX-2 in DU-145 cells. In addition, NF-κB is known to be inhibitory transcription factor of apoptosis. To investigate the hypothesis that MT can inactivate NF-κB and suppress the expression of inflammation related genes such as iNOS, COX-2 and $cPLA_2$, and thereby prevent anti-apoptotic ability of NF-κB causing DU-145 cells go apoptosis, I

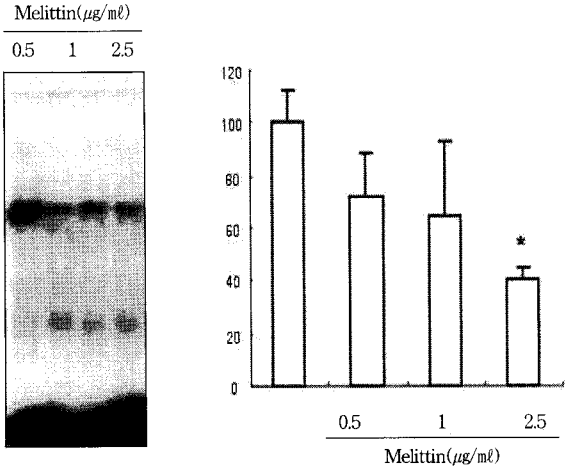

Fig. 3. Inhibitory effect of MT on NF-κB activity in DU-145 cells

* represented significant difference($p<0.05$), compared with control.

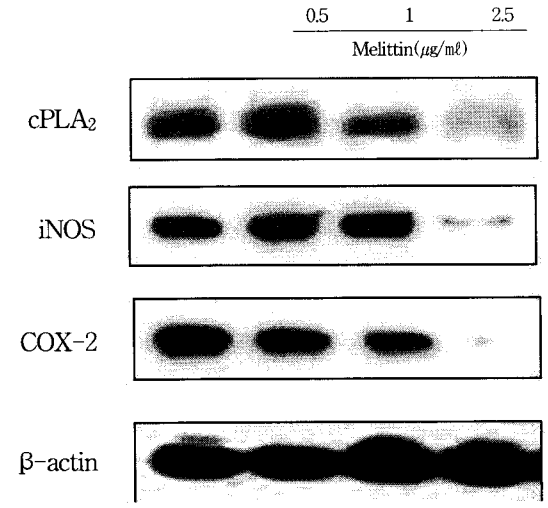

Fig. 4. Effects of MT on inflammatory gene expression in DU-145 cells

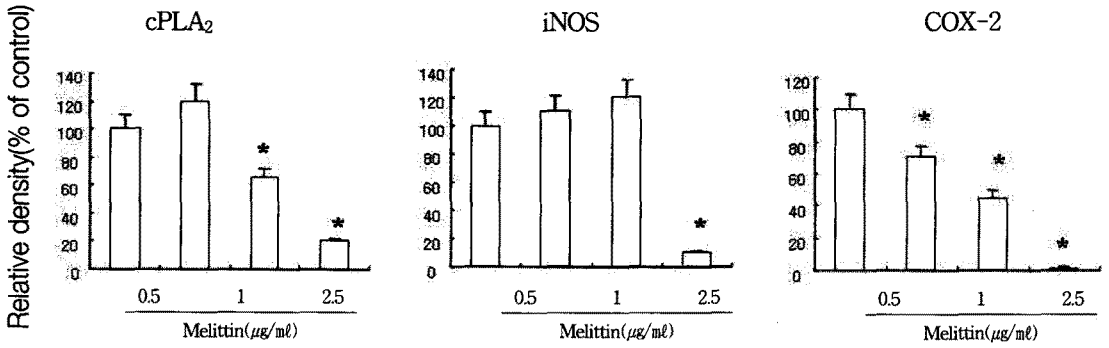

Fig. 5. Inhibitory effect of MT on inflammatory genes in DU-145 cells
* represents significant difference($P<0.05$) compared with control.

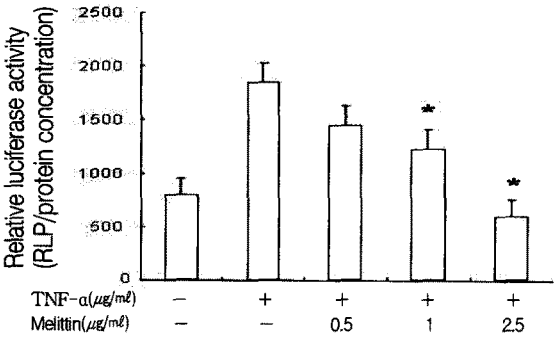

Fig. 6. Effects of MT on TNF-α induced NF-κB dependent luciferase activity in DU-145 cells

assessed NF-κB activity in the cells treated for different concentration of MT for 24hr by EMSA and observed inflammation gene expression in the cells by western blot analysis. NF-κB was highly activated in this cell and inflammation related gene expression was also enhanced, however the activation of NF-κB and inflammatory gene expression were gradually decreased by the culture in the presence of MT in the cells(Fig. 3-6). In NF-kB activity, the density of control significantly decreased by 2.5μg/mℓ of MT was 41.0±4.0%. In iNOS expression, the density of control significantly decreased by 2.5μg/mℓ of MT was 10.0±1.0%. In COX-2 expression, the density of control significantly decreased by 0.5, 1 and 2.5μg/mℓ of MT was 70.0±7.0, 45.0±4.5 and 1.0±1.0%. In $cPLA_2$ expression, the density of control significantly decreased by 1 and 2.5μg/mℓ of MT was 65.0±7.0 and 20.0±2.0%.

Activation of NF-κB was determined by electrophoretic mobility shift assay(EMSA), as described in Materials and Methods. Nuclear extracts from DU-145 cells with 0.5, 1 and 2.5μg/mℓ of MT were incubated in binding reactions of 32P-labeled oligonucleotide containing the B sequence. NF-κB DNA binding activity was determined by EMSA.

DU-145 cells were treated with 0.5, 1 and 2.5μg/mℓ of MT at 37°C for 24hours. Equal amounts of total proteins(80g/lane) were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and expression of cytosolic phospholipase A2(cPLA), cyclooxygenase 2(COX-2), inducible nitric oxide synthase(iNOS), and β-actin(as an internal control) was detected by Western blotting using specific antibodies. Quantification of band intensities from 3 independent experimental results was determined by densitometry.

4. Inhibitory effect of MT on NF-κB dependent luciferase activity in DU-145 cells

Transcriptional regulation involving the activation of NF-κB has been implicated in the expression of COX-2 and iNOS. To determine the role of MT in NF-κB dependent gene transcription, I conducted a transient transfection assay with a fusion gene containing SV40 promoter, 5 repeats of the consensus NF-κB binding sequence, and the luciferase reporter gene. DU-145 cells were transfected with

this promoter-reporter gene construct, and transcriptional activities were measured after TNF-α stimulation with or without MT. As shown in Fig. 6, cotreatment of the transfected cells with MT significantly inhibited the luciferase activity induced by TNF-αin DU-145 cells(Fig. 6).

DU-145 cells were transfected with pNF-κB-Luc plasmid (5NF-κB) and then activated with TNF-α in absence or presence of 0.5, 1 and 2.5μg/㎖ of MT for 2 hours, and then the luciferase activity was determined. Values are the mean and SEM of 3 independent experiments performed in triplicate. The level of induction was calculated relative to the luciferase activity in unstimulated transfected cells.

5. Mechanism of NF-κB inactivation

According to previous reports[12,13], Son et al.[13] demonstrated that Snake Venom Toxin(SVT) did not inhibit cell viability in PC-3 cells transfected with p50(C62S) mutant, and the abolished interaction between SVT and mutant p50 suggested that the removed cysteine residues are targets of SVT. However Lee et al.[12] MT or BV inhibited cell viability and NF-κB activity dose dependently in the LNCaP cells transfected with p50(C62S) mutant as well as in wild LNCaP cells. In the present study, To confirm whether MT also inhibits NF-κB through strong binding of MT to cysteine residues with sulfhydryl group in DU-145 cells, and to corroborate the above findings, transient transfection assay using a fusion gene containing pCMV promoter and p50 or IKKs mutant was conducted. DU-145 cells were transfected with these mutant genes constructs. NF-κB activity and Cell viability were then measured after stimulating the cells with MT(0.5, 1, 2.5μg/㎖) for 24hr. Contrary to the above son et al.'s and similar to Lee et al.'s, present study revealed that MT inhibited cell viability and NF-κB activity dose dependently in the DU-145 cells transfected with p50(C62S) or IKKs(IKKα(C178A), IKKβ(C179A), IKKβ(K44A)) mutant as well as in wild DU-145 cells or vector alone. The percentage of control significantly decreased by 2.5μg/㎖ of

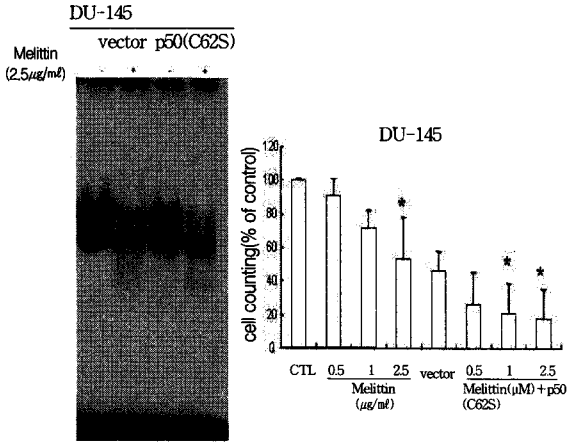

Fig. 7. Inactivation of NF-κB in the p50 mutant plasmid transfected DU-145 cells

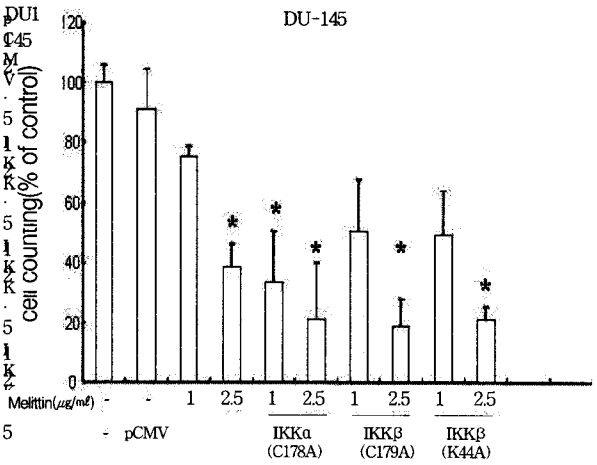

Fig. 8. Inactivation of NF-κB in the IKKs mutant plasmid transfected DU-145 cells

MT in the DU-145 cells was 61.3±39.2, which of vector significantly decreased by 0.5, 1 and 2.5μg/㎖ of MT in the DU-145 cells transfected with p50(C62S) mutant was 27.4±25.2, 24.2±22.4 and 21.1±26.9% respectively(Fig. 7), which of contol significantly decreased by 2.5μg/㎖ of MT in the vector alone was 39±2.6, which of vector control significantly decreased by 1 and 2.5μg/㎖ of MT in the DU-145 cells transfected with IKKα(C178A) mutant was 34.0±14.0 and 21.0±17.0, which of vector signifi- cantly decreased by 2.5μg/㎖ of MT in the DU-145 cells transfected with IKKβ(C179A) or IKKβ(K44A) was 19.0±9.7 or 21.0±7.8% respectively(Fig. 8).

Ⅳ. Discussion

The central and novel finding in the present study is the identification of anti-proliferation efficacy of MT against human testosterone refractory prostate carcinoma DU-145 cells. Most of the current available anti-cancer drugs exert their effect via promotion of apoptosis in cancer cells[14,15], which is regarded as one of the major mechanisms for the targeted therapy of various cancers including prostate cancer[14-17].

In case of prostate cancer, cancer cells become resistant to apoptosis and do not respond to cytotoxic chemotherapeutic agents by the transition of hormone sensitive cancer to hormone refractory one[16]. Therefore, the agents that induce apoptotic cell death of prostate cancer cells could be useful in controlling this hormone refractory malignant cancer[17]. Consistent with this approach, my data showing an induction of apoptotic cell death in prostate cancer control suggest that extremely low concentration(below 2.5μg/ml) of natural toxin(MT) could be useful as a candidate preventing and treating this malignancy.

It has been well established that NF-κB is an important element in regulating cell growth or apoptosis of tumor cells, including prostate cancer cells[18]. Recently several studies have reported that NF-κB is constitutively activated in human prostate cancer tissue, androgen-insensitive human prostate carcinoma cells, and prostate cancer xenografts[19,20]. Moreover, compounds inhibiting NF-κB have shown to induce apoptotic cell death of PC-3 cells[20,21]. Therefore, agents capable of suppressing NF-κB pathway may be potentially useful in the prevention and management of prostate cancer growth via induction of apoptotic prostate cancer cell death. In the present study, as DU-145 cells were exposed to MT(0.5-2.5μg), the cells were not grown, and died in a dose dependent manner. Moreover, MT inhibited prostate cancer cell growth significantly in 1 and 2.5μg/㎖ of MT. In addition to anti-proliferation effect of MT confirmed, characteristic apoptotic morphologic change of DU-145 cell was observed in change of the chromatin morphology of DU-145 cells using DAPI staining, implying inhibition of DU-145 cell growth was due to apoptosis.

In NF-κB activity of the cells treated with different concentration of MT for 24hr by EMSA, NF-κB was highly activated in DU-145 cells, while activation of NF-κB was gradually decreased by the culture in the presence of MT in the cells, and in the MT untreated DU-145 cells by western blot analysis, inflammation related gene expression was also enhanced, however it was gradually decreased by MT in the MT treated cells.

Meanwhile, transcriptional regulation involving the activation of NF-κB has been implicated in cancer growth as well as the expression of COX-2 and iNOS. To determine the role of MT in NF-κB dependent gene transcription, a transient transfection assay with a fusion gene containing SV40 promoter, 5 repeats of the consensus NF-κB binding sequence, and the luciferase reporter gene was conducted. DU-145 cells were transfected with this promoter-reporter gene construct, and transcriptional activities were measured after TNF-α stimulation with or without MT. Cotreatment of the transfected cells with MT significantly inhibited the luciferase activity induced by TNF-αin DU-145 cells. These data substantiated the hypothesis that MT can inactivate NF-κB and suppress the expression of inflammation related genes such as iNOS, COX-2 and $cPLA_2$, and thereby prevent anti-apoptotic ability of NF-κB causing DU-145 cells go apoptosis, suggesting that NF-κB signal may be significant contributor in MT-induced DU-145 cell death.

Much efforts have been so far made to elucidate the mechanism how natural toxins affects on the signal molecule involved in the NF-κB singnal pathway on the molecular basis.

According to Son et al[13], at the molecular level, SVT inhibited constitutively activated NF-κB signaling by impairing IκBα phosphorylation with inhibition of p50 translocation, and it bound with sulfhydryl group of cysteine residue in NF-κB, IKK α and IKKβ resulting in down regulation of NF-κB

activity, Moreover, abolished SVT-induced apoptotic cell death was found in the cells transfected with mutant p50, IKKα and β in which the cysteine residue was replaced with other amino acids.

In their transient transfection assay performed to ascertain whether promising interaction between SVT and p50 of NF-κB in PC-3 cells from the previous report[13] can be intactly applied to Bee venom or melittin interacting with p50 in LNCaP cells, Lee et al.[12] found that MT or BV inhibited LNCaP cells proliferation and NF-κB activation but unexpectedly inconsistent with the previous Son's report[13], the reverse effect was not actually shown in the LNCaP cells transfected with p50(C62S) mutant, and they surmised that anti-proliferation effect of MT or BV on LNCaP cells through induction of apoptosis came from another mechanism such as interaction with IKKs in the LNCaP cells. In the present study, to confirm whether MT also inhibits NF-κB through strong binding of MT to cysteine residues with sulfhydryl group in DU-145 cells, and to corroborate the above findings and Lee's assumption[12], transient transfection assay using a fusion gene containing pCMV promoter and p50 or IKKs mutant was conducted. DU-145 cells were transfected with these mutant genes constructs. NF-κB activity and Cell viability were then measured after stimulating the cells with MT(0.5, 1, 2.5μg/㎖) for 24hr. Contrary to the above son et al.'s and similar to Lee et al.'s, present study revealed that MT inhibited cell viability and NF-κB activity dose dependently in the DU-145 cells transfected with p50(C62S) or IKKs(IKKα(C178A), IKKβ(C179A), IKKβ(K44A)) mutant as well as in wild DU-145 cells or vector alone.

Consquently, although I could not elaborate on the reason why the abolition of son's report[13] was not reproduced in this study in spite of examining Lee's finding and assumption more concretely, and further mechanism study is thus needed, my present findings actually revealed that anticancer effect against advanced DU-145 human prostate cancer cells of MT is associated with down-regulation of the constitutively overexpressed or NF-κB-dependent anti-apoptotic proteins via reduction of nuclear translocation of NF-κB proteins, and they could form a basis for making MT available as a novel agent for human prostate cancer prevention or intervention without safety apprehension.

V. Acknowledgement

This study was supported by a grant of Kyungwon University.

VI. References

1. Hellerstedt BA, Pienta KJ. The current state of hormonal therapy for prostate cancer. CA Cancer J Clin. 2002 ; 52 : 154-79.
2. Feldman BJ, Feldman D. The develop- ment of androgenindependent prostate cancer. Nat. Rev. Cancer. 2001 ; 1 : 34-45.
3. Nelson WG, De Marzo AM, Isaacs WB. Prostate cancer. N Engl J Med. 2003 ; 349 : 366-81.
4. Santos AF, Huang H, Tindall DJ. The androgen receptor: a potential target for therapy of prostate cancer. Steroids. 2004 ; 69 : 79-85.
5. Srilatha Swami, Aruna V Krishnan, Donna M Peehl, David Feldman. Genistein potentiates the growth inhibitory effects of 1,25-dihydroxyvitamin D3 in DU145 human prostate cancer cells: Role of the direct inhibition of CYP24 enzyme activity. Molecular and Cellular Endocrinology 2005 ; 241 : 49-61.
6. Brown RL, Haley TL, West KA, Crabb JW. Pseudechetoxin: a peptide blocker of cyclic nucleotide-gated ion channels. Proc Natl Acad Sci USA. 1999 ; 96 : 754-9.
7. Sher E, Giovannini F, Boot J, Lang B. Peptide neurotoxins, small-celllung carcinoma andneurological paraneoplastic syndromes. Biochimie. 2000 ; 82 : 927-36.

8. van Lunteren E, Moyer M. Peptide toxin blockers of voltage-sensitive K+ channels: inotropic effects on diaphragm. J Appl Physiol. 1999 ; 3 : 1009-16.
9. Park HJ, Lee SH, Son DJ, Oh KW, Kim KH, Song HS, Kim GJ, Oh GT, Yoon DY and Hong JT. Antiarthritic effect of bee venom: inhibition of inflammation mediator generation by suppression of NF-kappaB through interaction with the p50 subunit. Arthritis Rheum 2004 ; 50 : 3504-15.
10. Holle L, Song W, Holle E, Wei Y, Wagner T and Yu X. A matrix metalloproteinase 2 cleavable melittin/avidin conjugate specifically targets tumor cells in vitro and in vivo. Int J Oncol. 2003 ; 22 : 93-98.
11. Park HJ, Lee YK, Song HS, Kim KJ, Son DJ, Lee JW and Hong JT. Melittin inhibits human prostate cancer cell growth through induction of apoptotic cell death. J. Toxicol. Pub. Health. 2006 ; 22(1) : 31-7.
12. HS Lee, HS Song. Bee venom inhibits LNCaP cell proliferation through induction of apoptosis via inactivation of NF-κB. Journal of acupuncture and moxibustion 2008 ; 25(2) : 59-74.
13. Dong Ju Son, Mi Hee Park, Sang Jin Chae et al. Inhibitory effect of snake venom toxin from Vipera lebetina turanica on hormone-refractory human prostate cancer cell growth: induction of apoptosis through inactivation of nuclear factor KB. Mol Cancer Ther. 2007 ; 6(2) : 675-83.
14. Lowe SW, Lin AW. Apoptosis in cancer. Carcinogenesis. 2000 ; 21 : 485-95.
15. Guseva NV, Taghiyev AF, Rokhlin OW, Cohen MB. Death receptor-induced cell death in prostate cancer. J Cell Biochem. 2004 ; 91 : 70-99.
16. Gurumurthy S, Vasudevan KM, Rangnekar VM. Regulation of apoptosis in prostate cancer. Cancer Metastasis Rev. 2001 ; 20 : 225-43.
17. Kantoff PW. New agents in the therapy of hormone-refractory patients with prostate cancer. Semin Oncol 1995 ; 22(1 Suppl 1) : 324.
18. Suh J, Payvandi F, Edelstein LC et al. Mechanisms of constitutive NF-kappaB activation in human prostate cancer cells. Prostate. 2002 ; 52 : 183-200.
19. Raj GV, Sekula JA, Guo R, Madden JF, Daaka Y. Lysophosphatidic acid promotes survival of androgen-insensitive prostate cancer PC3 cells via activation of NF-kappaB. Prostate. 2004 ; 61 : 105-13.
20. Syrovets T, Gschwend JE, Buchele B et al. Inhibition of IkappaB kinase activity by acetylswellic acids promotes apoptosis in androgen-ndependent PC-3 prostate cancer cells *in vitro* and *in vivo*. J Biol Chem. 2005 ; 280 : 6170-80.
21. Li Y, Sarkar FH. Inhibition of nuclear factor kappaB activation in PC3 cells by genistein is mediated via Akt signaling pathway. Clin Cancer Res. 2002 ; 8 : 2369-77.