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원 저

The Inhibitory Effects of Melittin on Human Prostate Cancer Cell PC-3 *in vivo and in vitro*

Yun Jong-il and Song Ho-sueb

Department of Acupuncture & Moxibustion, College of Oriental Medicine, Kyung Won University

국문초록

Melittin의 전립선암세포 증식에 대한 억제 효과

윤종일 · 송호섭

경원대학교 한의과대학 침구학교실

목적 : 이 연구는 봉독의 주요 성분인 낮은 농도의 melittin이 in vitro에서 세포자멸사 관련 단백질과 전립선암세포 PC-3 증식 관련 수용체의 발현 조절을 통하여 세포자멸사(Apoptosis)를 유도하는지 in vivo에서 또한 전립선 암세포주인 PC-3 세포의 성장을 억제하는지 살펴보고자 하였다.

방법 : Melittin을 처리한 후 전립선암세포 PC-3의 성장억제를 관찰하기 위해 WST-1 assay와 morphology analysis를 시행하였고, 세포자멸사 관련 MAP kinase 계열의 대표인 ERK1/2과 전립선암세포 증식관련 수용체인 PDGF-BB receptor β의 활성 변화 관찰에는 western blot analysis 및 Immunofluorescence Staining, Confocal immunocytochemistry를 시행하였으며, 전립암세포의 종양형성에는 흉선을 제거한 쥐에 Tumorigenecity study를 시행하였다.

결과 : 1. PC-3 세포에서 Melittin 처리 후 세포증식이 억제되었고, 세포의 형태는 세포자멸사의 특징을 나타내었다.

2. PC-3 세포에서 Melittin 처리 후 ERK1/2과 PDGF-BB receptor β의 활성이 억제되었다.

3. PC-3 세포에서 Melittin과 AG1296을 함께 투여시 PDGF-BB receptor β 활성억제의 상승효과가 나타났다.

4. 흉선 제거 후 전립선 암세포주를 이식한 쥐에서 Melittin을 피내로 주입한 결과 전립선암의 크기와 무게가 유의하게 감소하였다.

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· Corresponding author : Song Ho-Sueb, Director of Acupuncture & Moxibustion Department, College of Oriental Medicine, Kyungwon University Seoul Hospital, 20-8 Songpa-dong Songpa-gu Seoul city Republic of Korea
Tel. 02-425-3456 E-mail : hssong70@kyungwon.ac.kr

결론 : 이상의 결과는 Melittin이 ERK1/2과 PDGF BB receptor β의 활성 억제를 통하여 인간 전립선암 세포주인 PC-3의 세포자멸사를 유발함으로써 증식억제 효과가 있음을 입증한 것이며, 이를 재확인한 생체 연구에서의 긍정적인 결과는 향후 Melittin의 전립선암 예방과 치료에 대한 효과적인 치료제 개발에 초석이 될 것으로 기대된다.

핵심 단어 : melittin, prostate cancer, apoptosis, ERK1/2, PDGF-BB receptorβ, Xenograft, PC-3

Ⅰ. Introduction

Prostate cancer is the most common cancer as well as the second leading cause of cancer-related deaths in men of western countries[1]. One out of nine men over 65years of age is frequently diagnosed with prostate cancer in the United States[1,2]. Current therapies for prostate cancer, such as surgery, radical prostatectomy and radiation therapy, are effective in many patients with locally advanced disease, but many of these patients eventually have recurrence[3]. Cytotoxic chemotherapies do not show any significant improvement in patient condition, either due to the high resistance of prostate cancer cells against chemotherapeutic agents, which is responsible for 28,000 deaths per year[1,4]. In the normal prostate, organ homeostasis is maintained by a dynamic balance between the rate of cell proliferation and the rate of programmed cell death (apoptosis)[5]. Failure to undergo apoptosis has been implicated in tumor development and resistance to cancer therapy. Promotion of apoptosis in prostate cancer cell may lead to the regression of cancer cells, and improved prognosis of refractory disease[6,7]. Thus any agents inducing apoptosis may be useful for chemotherapy against prostate cancer[8].

Recent 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[9,10]. Although, it was reported that activation of NF-κB by lysophosphatidic acid promotes survival of PC-3 cells[9], Syrovets et al reported that inhibition of NF-κB activity by acetyl-boswellic acids promotes apoptosis in androgen-independent PC-3 cells in vitro and in vivo[10]. Li and Sarkar also reported that inactivation of NF-κB by soy isoflavone genistein contributes to increased apoptosis of human PC-3 cancer cells[11]. Kim et al also previously found that apoptotic cell death of neuroblastoma by peroxisome proliferators 15-deoxy-Delta12, 14-prostaglandin J2 was accompanied with inactivation of NF-κB[12]. Therefore, agents capable of suppressing NF-κB pathwaymay be potentially useful in the prevention and management of prostate cancer growth and resistance via induction of apoptotic prostate cancer cell death.

Elucidating important molecular mechanisms of specific toxin-receptor and/or ionchannel complexes have been largely studied in drug discovery using natural toxins[13-15]. Bee venom (BV) is known to be a very complex mixture of active peptides, including melittin(a major component of BV), phospholipase A2, apamin, adolapin, and mast cell-degranulating peptide (MCDP)[16]. Increasing studies have demonstrated that melittin inhibits cancer growth, and induction of apoptotic cell death[17]. Park et al reported that target inactivation of nuclear factor κB (NF-κB) by directly binding to the p50 subunit was an important mechanism of the antiarthritic effect of BV[18], and that melitttin 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[19]. Son et al also recently found that

melittin inhibited smooth muscle cell proliferation via induction of apoptotic cell death through down activation of NF-κB[20]. To investigate the effect of melittin on prostate cancer cell growth and to gain better insight into the action mechanism and to form a basis for the development of melittin as a novel agent for human prostate cancer prevention and intervention.

In this study, we conducted an in vitro and in vivo analysis to evaluate the prostate cancer cell response to melittin in order to determine the ability of this venom toxin as a therapeutic agent to suppress prostate cell growth and resistance by inducing apoptotic cell death, and determine possible mechanisms related with suppression of proliferation of PC-3 cells.

Ⅱ. Materials and Methods

1. Chemicals

Melittin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). PDGF-BB was obtained from Upstate Biotechnology(Lake Placid, NY, USA). ERK1/2, phospho-ERK1/2 and PDGF receptor β antibodies were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). β-actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). [3H]thymidine was from Amersham Pharmacia Biotech (Buckinghamshire, UK). The cell culture materials were obtained from Gibco-BRL(Rockville, MD, USA), and other chemical reagents were from Sigma Chemical Co.

2. Cell Culture

The PC-3 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 (100 unit/ml, Bioproducts, Walkersville, MD) Cell cultures were then maintained at 37℃ in a humidified atmosphere of 5% CO2

3. PC-3 cells proliferation assays

The human prostate cancer cell PC-3 proliferation was measured using by cell counting and DNA synthesis assay as previously described[21]. For cell counting, cells were seeded in 12-well culture plates at 1×105cells/ml, and cultured in DMEM with 10% FBS at 37℃ for 24 hours. The cells were then cultured with serum-free medium containing melittin(0.25 ~ 0.75μg/ml) or vehicle (DMSO). 24hours later, the cells were stimulated with 50ng/ml PDGF-BB, and then trypsinized with trypsin- EDTA and counted using a hemocytometer under microscopy.

DNA synthesis was assayed by measurement of the [3H]thymidine incorporation into cell DNA. Cells were seeded in 24-well culture plates under the same conditions. The medium was then replaced by serum-free medium containing melittin, or vehicle. 24hours later, cultures were then exposed to 50ng/ml PDGF-BB for 20hours before 2μCi/ml of [3H]thymidine was added to the medium. Four hours later, labeling reactions were terminated by aspirating the medium and subjecting the cultures to sequential washes on ice with PBS containing 10% trichloroacetic acid and ethanol/ ether (1 : 1, v/v). Acid-insoluble [3H]thymidine was extracted into 300μl of 0.5M NaOH per well, and this solution was mixed with 3ml scintillation cocktail (Ultimagold, Packard Bioscience Co., Meriden, CT, USA), and quantified using a liquid scintillation counter (model LS3801, Beckman, Düsseldorf, Germany).

4. Cell morphologic analysis

To observe the effects of melittin on cell morphology, cells were examined via phase-

contrast microscopy (ECLIPSE TE-300, Nikon Instech Co., Kawasaki, Kanagawa, Japan).

5. WST-1 assay

To evaluate the effect of melittin on PC-3 cell proliferation with or without U0126(ERK1/2 inhibitor) and/or AG1296(PDGF-BB receptor tyrosine kinase inhibitor) by 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 0.6µg/㎖ of melittin with or withoout U0126(ERK1/2 inhibitor) and/or AG1296(PDGF-BB receptor tyrosine kinase inhibitor). After treatment, Cell proliferation 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.

6. Western blot analysis

Western blot analysis was performed as previously described[22]. PC-3 cells were harvested and homogenized lysis buffer [50mM Tris(pH 8.0), 150mM NaCl, 0.02% sodium azide, 0.2% SDS, 1 mM phenylmethylsufonyl fluoride, 10μM aprotinin, 1% igapel 630(Sigma Chemical Co.), 10mM NaF, 0.5mM EDTA, 0.1mM EGTA and 0.5% sodium deoxycholate]. The cell extracts were centrifuged at 23,000×g for 10min. Equal amount of proteins (30μg) were separated on a SDS polyacrylamide gel, and then transferred to a nitrocellulose membrane (Hybond ECL, Amersham Pharmacia Biotech Inc.). Blots were blocked for 2 h at room temperature with 5% (W/V) non-fat dried milk in Tris-buffered saline [10mM Tris (pH 8.0) and 150 mM NaCl] solution containing 0.05% Tween-20. The membrane was washed and incubated with phospho-p44/42 MAP Kinase (ERK1/2), PDGF receptor β antibodies at 1 : 500 dilution in BSA/ TTBS-T buffer for over night at 4°C and horseradish peroxidase- conjugated IgG secondary antibody (Santa Cruz Biotechnology Inc.) at 4°C over 3h. Immunoreactive proteins were detected with the ECL Western blotting detection system. The relative density of the protein bands was scanned by densitometry using MyImage (SLB, Seoul, Korea), and quantified by Labworks 4.0 software (UVP Inc., Upland, CA, USA).

7. Immunofluorescence Staining

PC-3 cells were plated in chambered tissue culture slides at a density of 2 × 103cells/well in DMEM. The cells were then cultured with serum-free medium containing melittin (0.5μg/ml) or vehicle. 24hours later, the cells were stimulated by 50ng/ml PDGF-BB for 1h, and then washed once with PBS and fixed with 4% paraformaldehyde for 20min, membrane- permeabilized by exposure for 2min to 0.1% Triton X-100 in phosphate-buffered saline, and placed in blocking serum(5% bovine serum albumin in phosphate-buffered saline) at room temperature for 1h. The cells were then exposed to primary rabbit monoclonal p-PDFG- beta antibody(1 : 250 dilution) overnight at 4°C, After washes with ice-cold PBS followed by treatment with an anti-goat biotinylated secondary antibody Alexa Fluor 568(Molecular Probes Inc., Eugene, OR, USA), 1 : 200 dilution, for 4h at room temperature. Nuclear stain and mount in antifade medium with DAPI (Vector Laboratory Inc.), immunofluorescence images were acquired using a confocal laser scanning microscope (TCS SP2, Leica Microsystems AG, Wetzlar, Germany) equipped with a 630× oil immersion objective.

8. Tumorigenecity studies in athymic nude mice

For present study, we employed PC-3 human prostate cancer cells because they develop rapid tumors with high secretion of prostate- specific antigen(PSA) in the host. A total of 1 × 106cells

suspended in 50㎕ of media and 50㎕ of Matrigel (BD Biosciences, Bedford, MA) were inoculated s.c. into the right flank of sixteen 6-week-old mice by using a 27-gauge needle and divided into control and melittin groups which were 3mg/kg (75μg/100㎕ saline/25g mouse) of melittin group (melittin 3) and 6mg/kg(150μg/100㎕ saline/25g mouse) of melittin group(melittin 6). Whereas melittin group of animals received an i.d. injection of 75μg/100㎕ or 10μg/100㎕ of saline every three days, the second group received an i.p. injection of 100㎕ saline alone and served as the control group. Every week, tumor growth was estimated in terms of volume of tumors (㎣) as a function of time (days).

9. Statistical analysis

Data were analyzed using one-way analysis of variance followed by Tuckey test as a post hoc test(Sigma Plot software; SPSS, Chicago, IL, USA). Differences were considered significant at $p<0.05$, $p<0.01$.

Ⅲ. Results

1. Inhibitory effects of Melittin on proliferation of PC-3 cells

We first sought to determine whether melittin can inhibit the proliferation of human prostate cancer PC-3 cells. The inhibitory effects of melittin on the proliferation of PC-3 cells were examined by direct cell counting and DNA synthesis assay. The cell number was significantly increased by treatment with 50ng/ml PDGF-BB for 24h, and decreased significantly in a concentration-dependent manner by 24h pre- treatment with melittin. The percentages of the control cell number significantly decreased by melittin 0.25μg/ml, 0.5μg/ml and 0.75μg/ml were 75.4 ± 3.4, 62.4 ± 7.0 and 35.2 ± 5.0% on PDGF- BB-stimulated cells (Fig. 1).

Effects of melittin on DNA synthesis in rat aortic PC-3 cells were tested using [3H]thymidine incorporation. As shown in Figure 2, melittin concentration-dependently inhibited [3H]thymidine incorporation induced by PDGF-BB. The percentages of the control significantly decreased by melittin 0.25μg/ml, 0.5μg/ml and 0.75μg/ml were 62.2 ± 3.4, 42.4 ± 4.8 and 28.0 ± 4.8% on PDGF-BB-stimulated cells (Fig. 2). The inhibitory effects were also dependent on concentration and corresponded with the inhibition of cell number. Taken together, these results indicate that melittin significantly inhibit PC-3 cells proliferation, and melittin especially exhibited the strongest inhibition against PDGF-BB-induced PC-3 cells proliferation.

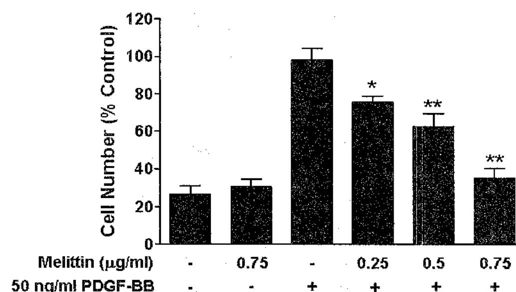

Fig. 1. Effect of Melittin on Cell Number in PC-3 cells

Cells were pre-treated with 0.25~0.75μg/ml melittin for 24h, and then stimulated with 50ng/ml PDGF-BB. $*P<0.05$ $**P<0.01$.

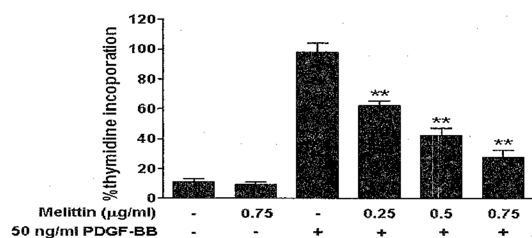

Fig. 2. Effect of Melittin on DNA Synthesis in PC-3 cells

Cells were pre-treated with 0.4~0.8μg/ml melittin for 24h, and then stimulated with 50ng/ml PDGF- BB. $*P<0.05$ $**P<0.01$.

2. Effect of Melittin on the morphologic change of PC-3 cells

Given the potent inhibition on cell proliferation by melittin, we evaluated PC-3 cells apoptosis by cell morphologic analysis.

To observe the effects of melittin on cell morphology, cells were examined via phase-contrast microscopy(ECLIPSE TE-300, Nikon Instech Co., Kawasaki, Kanagawa, Japan). In the morphologic analysis, melittin were seen to cause characteristic changes of PC-3 cells. Under the phase-contrast microscope, melittin-treated PC-3 cells for 24h presented with cytoplasmic blebbing, cell shrinkage, cytoplasmic condensation, and irregularity in shape(Fig. 3). These morphological characteristics suggest that melittin induce apoptotic cell death in PC-3 cells.

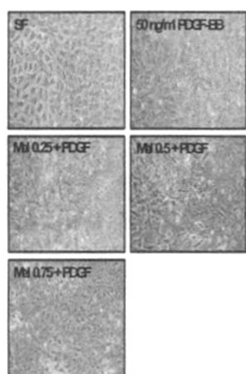

Fig. 3. Effect of melittin on cell morphological changes in PC-3 cells
Treatment of melittin for 24h caused apoptosis characterized by cytoplasmic blebbing, cell shrinkage, cytoplasmic condensation, and irregularity in shape (phase-contrast microscopy magnification, × 200).

3. Inhibitory Effects of MElittin on PDGF-BB receptor β and ERK1/2 activation

To investigate the mechanisms of the anti-proliferative and pro-apoptotic effects exerted by melittin, we examined whether melittin could reduce the PDGF-BB-induced phosphorylation of PDGF-BB receptor β and ERK1/2. Pre-treatment of 0.25 μg/ml, 0.5μg/ml and 0.75μg/ml melittin significantly inhibited the PDGF-BB-induced phosphorylation of PDGF-BB receptor β in a concentration-dependent manner. The percentages of the control significantly decreased by melittin 0.5μg/ml and 0.75μg/ml were 125.6±19.2 and 74.7±30.0 % respectively(Fig. 4).

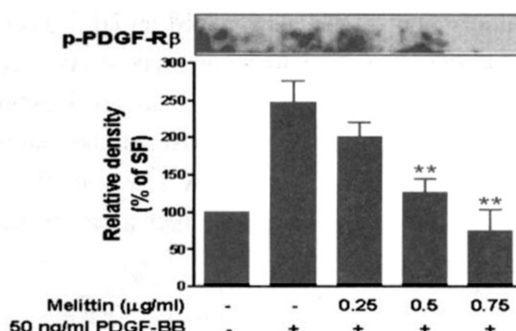

Fig. 4. Effect of Melittin on Expression of PDGF-BB receptor β in PC-3 cells
Cells were pre-treated with 0.25~0.75μg/ml melittin for 24h, and then stimulated with 50ng/ml PDGF-BB. Equal amounts of whole cell lysate (30μg) were subjected to electrophoresis and analysis by Western blot for total and phosphorylated PDGF-BB receptor β. Density of immunoblotting bands of phosphorylated PDGF-BB receptor β was measured as described under Materials and Methods. **: P<0.01, significantly different from the PDGF- BB-treated cells.

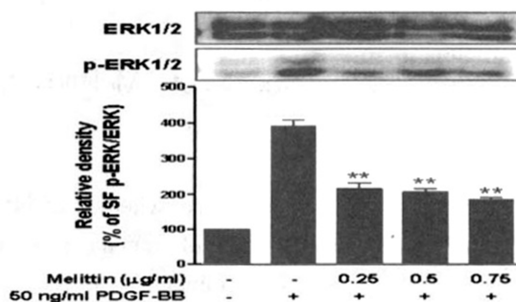

Fig. 5. Effect of Melittin on Expression of ERK1/2 in PC-3 cells
Cells were pre-treated with 0.25~0.75μg/ml melittin for 24h, and then stimulated with 50ng/ml PDGF-BB. Equal amounts of whole cell lysate (30μg) were subjected to electrophoresis and analysis by Western blot for total and phosphorylated ERK1/2. Density of immunoblotting bands of phosphorylated EKR1/2 was measured as described under Materials and Methods. *P<0.05 **P<0.01.

Melittin also inhibited 50ng/ml PDGF-BB-induced phosphorylation of ERK1/2 in a concentration-dependent manner. The percentages of the control decreased by melittin 0.25μg/ml, 0.5μg/ml and 0.75 μg/ml were 215.2 ± 16.4, 206.6 ± 10.1 and 185.7 ± 5.9%(Fig. 5).

To reconfirm anti-proliferative effect of melittin and to further demonstrate the involvement of PDGF-BB receptor β and ERK1/2 in human prostate cancer PC-3 cell death by melittin, we employed U0126 as a inhibitor of ERK1/2, which was expected to abrogate interaction between melittin and ERK1/2, thereby restore ERK1/2 activity from melittin-induced inactivation. inconsistent with restoring ERK1/2 activity, U0126 didn't actually abrogate melittin-induced PC-3 cell death dose dependently. While Percentages of the control significantly decreased by melittin 0.6μg/ml was 1.8 ± 0.1 O.D., That of melittin 0.6μg/ml with 10 or 20μM of U0126 were 1.8 ± 0.1 or 1.7 ± 0.1 O.D. respectively. The result indicated that inhibition of ERK1/2 activity might have little anti-proliferative influence upon PC-3 cells. We also used AG1296 as a PDGF-BB receptor tyrosine kinase inhibitor, which was expected to make the anti-proliferative and pro-apoptotic effect stronger. consistent with inactivation of PDGF-BB receptor AG1296 showed dose dependent synergic effect with melittin on proliferation of PC-3 cells. The percentages of melittin 0.6μg/ml with 10 or 20μM of AG1226 and U0126 were 0.4 ± 0.1 or 0.3 ± 0.1 O.D. respectively(Fig. 6).

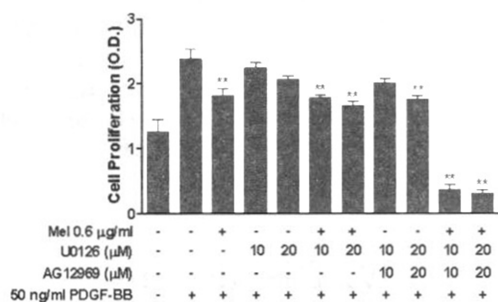

Fig. 6. Effect of Melittin on PDGF-BB receptor β phosphorylation

To evaluate the effect of melittin on PC-3 cell proliferation with or without U0126(ERK1/2 inhibitor) and/or AG1296(PDGF-BB receptor tyrosine kinase inhibitor) by WST-1 assay. ** represents significantly different from the PDGF-BB-treated cells(P<0.01).

4. Inhibotory Effects of Melittin on Expression of PDGF-BB receptor β in PC-3 cells

We also performed confocal laser-scanning microscopy analysis to demonstrate clearly the effect of melittin on PDGF-BB receptor β activation in situ in PC-3 cells. Treatment of melittin (0.25, 0.5μg/ml) decreased the number of PDGF-BB receptor β activated cells(Fig. 7).

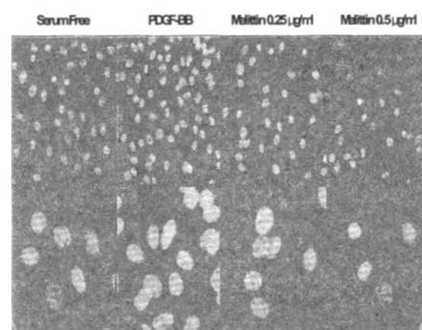

Fig. 7. Inhibition of Expression of PDGF-BB receptor β in PC-3 cells

Cells were examined under control condition after being stimulated by 50ng/ml PDGF-BB or pretreatment with 0.25, 0.5μg/ml of melittin for 24h. PC-3 cells were specifically stained for Phospho-PDGF-receptor β(red) and nuclear (blue). Laser Scanning Confocal Microscopy was performed with magnification (630×).

5. Effect of Melittin on Tumorigenecity of PC-3 prostate cancer cells in an athymic nude mouse model

Because melittin was observed to be effective in inhibiting the growth of PC-3 human prostate cancer cells in vitro, we next investigated whether these results could be translated into an in vivo xenograft model. melittin did not cause any loss in the body weight(Fig. 8), food intake, or exhibited

apparent signs of toxicity in animals. Implantation of PC-3 cells onto nude mice produced visible tumors in mice with a mean latent period of 18 days. The average volume and weight of tumors in control mice increased as a function of time and reached a preset end point of 0.87㎖ and 1.33g in 48days after inoculation. However, at this time, the average tumor volume and weignt of melittin 3 and melittin 6 was decreased in a dose dependent manner, which was 0.69㎖ and 0.9g, and 0.81㎖ and 0.72g respectively(Fig. 8). We also evaluated whether or not i.d .Injection of melittin into mice leads to inhibition of PC-3 prostate cancer cell growth in nude mice. Compared with control, Tumor growth inhibition ratio of melittin 3 and melittin 6 was about 42 and 45% in 48 days after inoculation(Fig. 9).

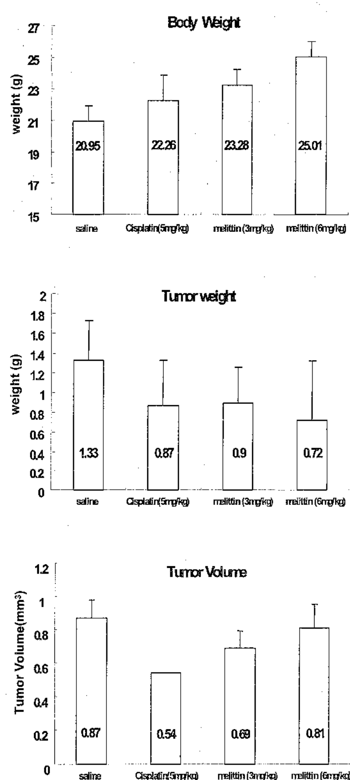

Fig. 8. Effect of Melittin on Volume and Weight of PC-3 cancer in a Xenograft nude mice

A total of 1×106cells suspended in 50$\mu\ell$ of media and 50$\mu\ell$ of Matrigel(BD Biosciences, Bedford, MA) were inoculated s.c. into the right flank of sixteen 6-week-old mice by using a 27-gauge needle and divided into control and melittin groups which were 3mg/kg(75μg/100$\mu\ell$ saline/25g mouse) of melittin group(melittin 3) and 0.4mg/kg(10μg/100$\mu\ell$ saline/25g mouse) of melittin group(melittin 0.4). Whereas melittin group of animals received an i.d. injection of 5μg/100$\mu\ell$ or 10μg/100$\mu\ell$ of saline every three days, the second group received an i.p. injection of 100$\mu\ell$ saline alone and served as the control group. Every week, tumor growth was estimated in terms of volume and weight of tumors (㎣ and g) as a function of time.

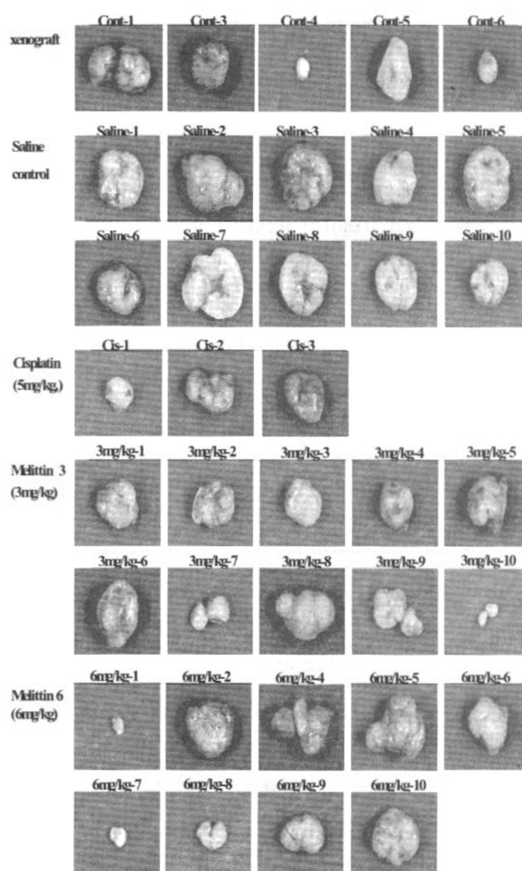

Fig. 9. Tumor Mass extracted from the Saline (Control), melittin 3, melittin 6

These are photographs of tumor mass taken out of 10 PC-3 xenograft nude mice in the control group, 9 PC-3 xenograft nude mice treated with low dose(3 mg/Kg) of melittin in the melittin 3 group and 10 PC-3 xenograft nude mice treated with high dose(6 mg/Kg) of melittin in the melittin 6 group after sacrifice. one in the melittin 3 was passed away.

Ⅳ. Discussion

The central and novel finding in the present

study is the identification of in vitro and in vivo anticancer efficacy of melittin from Bee venom against advanced human prostate carcinoma PC-3 cells. Most of the present available cytotoxic anticancer drugs mediate their effect via induction of apoptosis in cancer cells[22,23], and induction of apoptosis is suggested as one of the major mechanisms for the targeted therapy of various cancers including prostate cancer[22-25]. In case of advanced prostate cancer, cancer cells become resistant to apoptosis and do not respond to cytotoxic chemotherapeutic agents[4]. The anti-proliferative effects of melittin on cancer cell growth via apoptotic cell death have been reported in several cancer cells including hepatocarcinoma cells[26], breast cancer cells[27], and osteocarcinoma cells[28]. Therefore, the agents that induce apoptotic cell death of prostate cancer cells could be useful in controlling this malignancy[29]. Consistent with this approach, our data showing an induction of apoptotic cell death in prostate cancer control suggest that extremely low concentration (below 1μg/ml) of natural toxin (melittin) could be useful as a anti-cancer agent.

According to recent reports elucidating (son et al, park et al) the mechanism of anti-proliferation of cells such as rat aortic vascular smooth muscle cell(VSMC) and human prostate cancer cell(PC-3), Son et al. found that melittin potently inhibited PDGF-BB induced phosphorylation and degradation of IκB and markedly suppressed activation of NF-κB and phosphorylation of Akt and weakly inhibited phosphorylation of ERK1/2, and that Melittin also increased expression of pro-apoptotic protein p53, Bax, and caspase-3 and decreased antiapoptotic protein Bcl-2.

Park et al demonstrated that melittin acted as a very similar mechanism in PC-3 cells as found in RAW264.7 cells as well as VSMCs, and that activation of MAP kinase(especially ERK) pathway mignt be also involved in the melittin induced prostate cancer cell death. they emphasized that melittin induced apoptotic cell death of PC-3 Cells, and the alteration of the expression of apoptosis regulatory proteins resulting in a shift the cells favoring apoptotic cell death[19].

Oliver et al. clearly indicated that an acute and marked alteration in the tyrosine phosphorylation was related with prostate cancer cell[30].

Consistent with the previous studies, our data also suggested that melittin play a major role in anti-proliferation of human prostate cancer PC-3 cells via suppression of phosphorylation of ERK1/2 and PDGF-BB receptor β in vitro, and inhibition of tumor growth in vivo in a PC-3 xenograft nude mice as well.

Thus, our present findings showing the in vitro and in vivo anticancer efficacy of melittin, with mechanistic rationale including apoptosis induction, against advanced human prostate cancer cells and preclinical human prostate cancer models could confirm a basis for the development of melittin as a novel agent for human prostate cancer prevention and/or intervention without safety apprehension.

V. References

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