Original Article

Effect of Snake Venom Toxin from *Vipera lebetina turanica* on Breast Cancer Cells

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국문초록

Vipera lebetina turanica 사독이 인간 유방암 세포에 미치는 영향

양가람 · 송호섭

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목적 : 이 연구는 Vipera lebetina turanica의 蛇毒藥鍼液(Snake venom toxin, SVT)이 인간 유방암 세포주인 MCF-7과 MDA-MB-231 세포에서 암세포성장의 억제 및 그 기전에 대하여 살펴보고자 하였다.

방법 : SVT를 처리한 후 MCF-7과 MDA-MB-231의 성장억제를 관찰하기 위해 CCK-8 assay를 시행하였고, apoptosis 평가에는 TUNEL assay를 시행하였다. 세포자멸사 관련 세포기전을 보기 위하여 세포내 활성산소량 및 미토콘드리아의 세포막전위 변화를 측정하였고, 세포자멸사 조절 단백인 Bax, Bcl-2 발현 변화 관찰에는 western blot analysis를 시행하였다.

결과 : MCF-7과 MDA-MB-231 세포에 SVT를 처리한 후, 유방암 세포의 성장, Apoptosis의 유발 및 기전에 미치는 영향을 관찰하여 다음과 같은 결과를 얻었다.

1. MCF-7 세포와 MDA-MB-231 세포에서 SVT를 처리한 후 유방암 세포 성장이 억제되었다.
2. TUNEL assay를 통한 세포자멸사 평가에서 SVT를 처리한 MCF-7세포와 MDA-MB-231 세포 모두 세포자멸사 활성세포의 유의한 증가를 나타내었다.
3. 세포자멸사 관련 세포기전연구에서 SVT를 처리한 MCF-7 세포와 MDA-MB-231 세포에서 세포내 활성산소의 유의한 증가와 미토콘드리아 세포막 전위의 유의한 변동이 관찰되었다.
4. SVT를 처리한 MCF-7세포와 MDA-MB-231세포는 세포자멸사 관련 단백 발현에서 Bax의 유의한 증가와 Bcl-2의 유의한 감소를 나타내었다.

결론 : 이상의 결과는 SVT가 세포내 활성산소를 증가시킴으로써 미토콘드리아의 세포막전위에 변화를

· Acceptance : 2009. 5. 18. · Adjustment : 2009. 6. 4. · Adoption : 2009. 6. 4.
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일으켜 유방암 세포주인 MCF-7과 MDA-MB-231 세포에 세포자멸사를 유발하여 증식억제 효과가 있음을 입증한 것이다.

핵심단어 : 사독, 유방암, MCF-7, MDA-MB-231, 세포자멸사, 활성산소, MPT, Bcl-2, Bax

Ⅰ. Introduction

Breast cancer is a leading cause of morbidity and mortality in women, in developed and increasingly also developing countries[1,2]. This pathology is currently controlled by surgery and radiotherapy, and is frequently supported by adjuvant chemo- or hormonotherapies[3]. However, breast cancer is highly resistant to radiation and conventional chemotherapeutic agents, and this resistance is associated with a poor prognosis for this metastatic disease, especially in cases of hormone-independent cancer[3,4]. About 30%-40% of women with this form of cancer will develop metastases and eventually die of their disease[5]. Novel therapies are therefore needed to deal with the increasing incidence of human breast cancer.

The redox status of all aerobic cells is balanced by enzyme and nonenzyme systems[6-9]. Oxidative stress occurs when this critical balance is disrupted because of excess ROS production and/or antioxidant depletion[8,9].

Cytotoxic ROS signaling appears to be triggered by the activation of the mitochondrial-dependent cell death pathway through the proapoptotic Bcl-2 proteins Bax, with subsequent mitochondrial membrane permeabilization and cell death[6,10-12].

A snake venom toxin(SVT) from *Vipera lebetina turanica*, is a group of basic peptides, and important factor V activator composed of 236 amino acids with six disulfide bonds formed by twelve cysteins[13]. Shim et al.[14] reported that SVT induced apoptosis through significant increase of ROS generation and mitochondrial membrane potential in neuroblastoma cells. In this study, to investigate the effect of SVT from *Vipera lebetina turanica* on two highly invasive MCF-7(Estrogen dependent) and MDA-MB-231(Estrogen independent) breast cancer cell growth and to contribute to make SVT available as a novel agent for human breast cancer prevention and intervention, I therefor conducted a mechanism study involving ROS generation, mitochondrial membrane potential and proapoptotic Bcl-2 family, determining whether SVT exerts inhibitory effect on MCF-7(Estrogen dependent) and MDA-MB-231(Estrogen independent) cells proliferation, and how it works on the their apoptotic cell death.

Ⅱ. Materials and methods

1. Materials

Snake Venom(SVT) from *Vipera lebetina turanica* was purchased from Sigma Chemical Co. (Saint Louis, USA).

2. Cell culture

The Estrogen Receptor(ER) positive MCF-7 or ER negative MDA-MB-231 human breast cancer cell was obtained from ATCC(American Type Culture Collection, Rockville, MD). Breast cancer 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/㎖, Bioproducts, Walkersville, MD). Cell cultures were then maintained at

37℃ in a humidified atmosphere of 5% CO_2.

3. Cell viability assay

CCK-8 assay

Cells were plated at a density of 1×10^4 cells/well in 96-well plates and then subconfluent cells were exposed to different doses(1.25−20㎍/㎖) of SVT from *Vipera lebetina turanica*(Sigma, St. Louis, USA) for 6, 12, 24hr. After treatment, cell viability was measured by Cell Counting Kit-8(CCK-8) system(Dojindo Laboratory, Kumamoto, Japan) according to the manufacturer's instructions. CCK-8 solution was added to cells in 96-well plates, cells were incubated at 37°C for 1hr, and the optical density of each well was read at 450nm.

4. TUNEL staining and quantification

Adherent MCF-7 or MDA-MB-231 cells (10^6) were detached from culture dishes with 0.02% EDTA, and washed twice in PBS, prior to being fixed 1hr in 4% paraformaldehyde, 4°C, and permeablized with 0.1% Triton X-100 in 1% sodium citrate for 5min, 4°C. Apoptosis was determined by detecting DNA strand breaks with a TUNEL assay, using the *In Situ* Death Detection Kit(Boehringer Mannheim, Indianapolis, IN). Ninety seven percent of cells initially plated were recovered for analysis. The percentage of TUNEL-positive cells was determined with FACS analysis, using the Consort-40 software(Becton Dickinson). Cell populations were gated to a negative control in which neuronal cells were treated with labeling solution alone containing fluorescein-labeled dUTP but no terminal deoxynucleotidyl transferase.

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㎕/㎖ aprotinin, 1% igapel 630(Sigma-Aldrich, St. Louis, 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 Bax, Bcl-2 (Santa Cruz, 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 protein bands was scanned by densitometry using MyImage(SLB, Seoul, Korea), and quantified by Labworks 4.0 software (UVP Inc, Upland, California).

6. Measurement of mitochondrial membrane potential

The loss of mitochondrial membrane potential (MMP) is a hallmark for apoptosis. It is an early event preceding phosphotidylserine externalization and coinciding with caspases activation. MMP was measured using JC-1 kit following the protocol submitted by supplier. The mitochondrial membrane potential detection kit uses a unique fluorescent cationic dye, JC-1(5,5'6,6'-tetracholoro-1,1',3,3'-tetraethyl-benzimidazolylcarbocyanine iodide) (excitation, 488nm and emission, 525nm), to signal the loss of MMP. Cells were harvested by trypsinization at 0.25 to 24hr of treatment with 10㎍/㎖ of SVT. Then mitochondrial permeability transition was determined by staining of the cells with JC-1 as described. Briefly, equal numbers of cells(1×10^6) were incubated with JC-1 at 2.5㎍/㎖ in 1㎖ PBS for 30min at 37℃ with moderate shaking. Cells were then centrifuged at 300g, 4℃ for 5min, washed twice with ice-cold PBS and finally

resuspended in 200㎕ PBS. Mitochondrial permeability transition was subsequently quantified on FACS(Becton Dickinson, San Diego, CA, USA). Data are given in % of cells with altered mitochondrial membrane potential.

7. Measurement of intracellular ROS level

MCF-7 or MDA-MB-231 cells were seeded into 100mm dishes(5×10^6 cells per dish) overnight. Cells were treated with 1.25-20㎍/㎖ of SVT for 0.25 to 24hr, and then collected by trypsinization. 200㎕ of cell suspension containing 2×10^6 cells/㎖ was added to 800㎕ of 10 PBS and was incubated with 2', 7'-dichlorofluorescein di-acetate(DCFH-DA) at 10μM concentration for 15mins. H_2O_2(25μM) and SVT(0, 1.25, 2.5, 5, 10, 20㎍/㎖) was added in the culture and the incubation was continued for an additional 20min at 37℃.

The production of intracellular H_2O_2 was measured using fluoremeter(excitation, 365nm and emission, 430nm). Another set of experiment was performed simultaneously, with same treatments and conditions, where catalase(50U/㎖) was added to the cell culture medium 5min before the addition of different concentrations of SVT.

8. 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 MCF-7 or MDA-MB-231 cell growth

To evaluate an effect of SVT from *Vipera lebetina turanica* on the cell growth of human breast cancer MCF-7 or MDA-MB-231 cells, I analyzed cell viability using CCK-8 assay, direct cell counting according to time course. SVT inhibited MCF-7 cell growth in a dose dependent manner at 6 and 24hr. The percentage of control significantly decreased by 5,10 and 20㎍/㎖ of SVT at 6hr was 0.94±0.02, 0.97±0.01 and 0.89±0.03%, which by 1.25, 2.5 and 20㎍/㎖ of SVT at 12hr was 0.97±0.02, 0.95±0.02 and 0.92±0.08%, which by 1.25, 2.5, 5, 10 and 20㎍/㎖ of SVT at 24hr was 1.05±0.02, 1.06±0.03, 1.03±0.02, 0.92±0.03 and 0.88±0.03%(Fig. 1). SVT also inhibited MDA-MB-231 cell proliferation dose dependently at 6, 12 and 24hr.

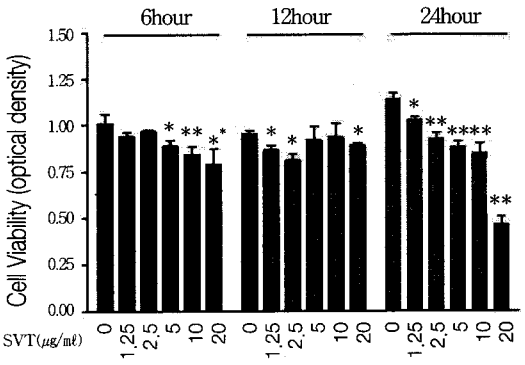

Fig. 1. Cell growth of MCF-7 cells by SVT

Cell viability was determined by CCK-8 assay as described under Method.

* or ** represent $P<0.05$ or $P<0.01$ significant compared with control respectively.

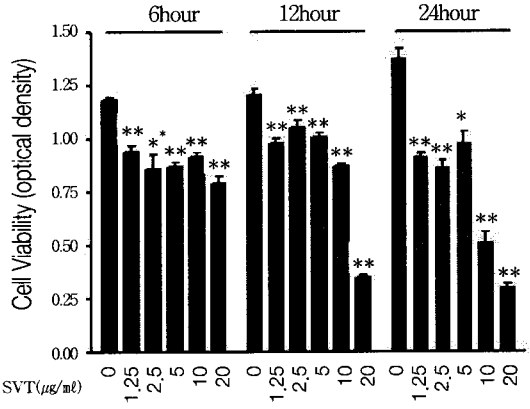

Fig. 2. Cell growth of MDA-MB-231 cells by SVT

Cell viability was determined by CCK-8 assay as described under Method.

* or ** represent $P<0.05$ or $P<0.01$ significant compared with control respectively.

The percentage of control significantly decreased by 1.25, 2.5, 5, 10 and 20μg/mℓ of SVT at 6hr was 0.94±0.03, 0.86±0.07, 0.87±0.02, 0.91±0.02 and 0.79±0.04%, which at 12hr was 0.98±0.02, 1.05±0.03, 1.00±0.03, 0.87±0.01 and 0.35±0.02%, which at 24hr was 0.91±0.02, 0.86±0.03, 0.98±0.05, 0.51±0.05 and 0.30±0.02%(Fig. 2).

2. Induction of apoptosis

To delineate whether the inhibition of cell growth by the SVT was due to the induction of apoptosis, I evaluated apoptosis of MCF-7 or MDA-MB-231 cells by TUNEL assay and quantification using FACS. TUNEL-positive MCF-7 or MDA-MB-231 cells were increased dose dependently. MCF-7 cells demonstrated significant increase in the apoptosis rate induced by 10 and 20μg/mℓ of SVT which was 16.7±3.1 and 41.3±4.1% respectively. MDA-MB-231 cells also showed significant increase of apoptosis rate in 2.5, 5, 10 and 20μg/mℓ of SVT which was 18.4±2.4, 23.6±2.3, 48.7±8.7 and 73.8±3.1% respectively(Fig. 3).

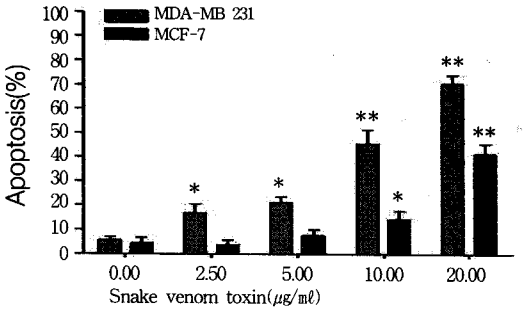

Fig. 3. Apoptosis of MCF-7 or MDA-MB-231 cells by SVT

Cell apoptosis of MCF-7 or MDA-MB-231 was determined by Tunel Assay and FACS Quantification after treatment as described under Method.

* or ** represent P<0.05 or P<0.01 significant compared with control respectively.

Moreover to elucidate the celllular mechanism of apoptosis involved in the cells by SVT, I also investigated the effect of ROS on the mitochondria measuring ROS generation level and mitochondrial membrane potential. In MDA-MB-231 cells, SVT induced ROS generation was significantly increased and reached 1.30±0.11 and 1.35 in 10 and 20μg/mℓ of SVT at 0.5hr, 1.30±0.11 in 10μg/mℓ of SVT at 1hr, and 1.21±0.11, 1.40±0.05 and 1.30±0.05 in 1.25, 2.5 and 10μg/mℓ of SVT at 3hr respectively(Fig. 4), whereas MCF-7 cells did not have any significant change except that 20μg/mℓ of SVT-induced ROS generation reached 1.44±0.13 with significance at

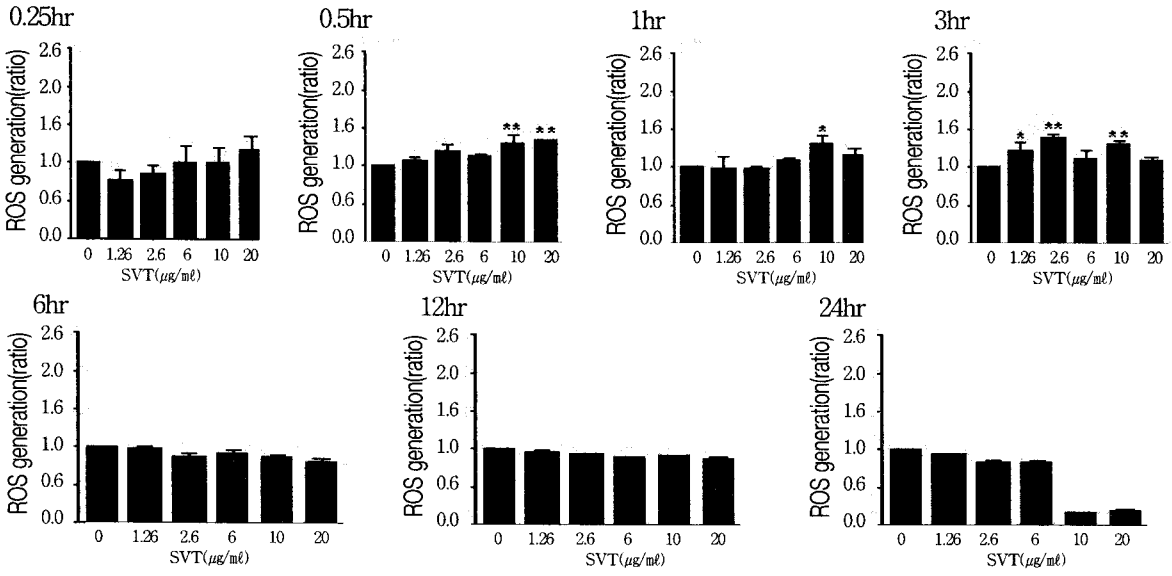

Fig. 4. ROS generation of MDA-MB-231 cells by SVT according to a time course

ROS generation of MDA-MB-231 cells was determined by ROS generation assay according to a time course as described under Method.

* or ** represent P<0.05 or P<0.01 significant compared with control respectively.

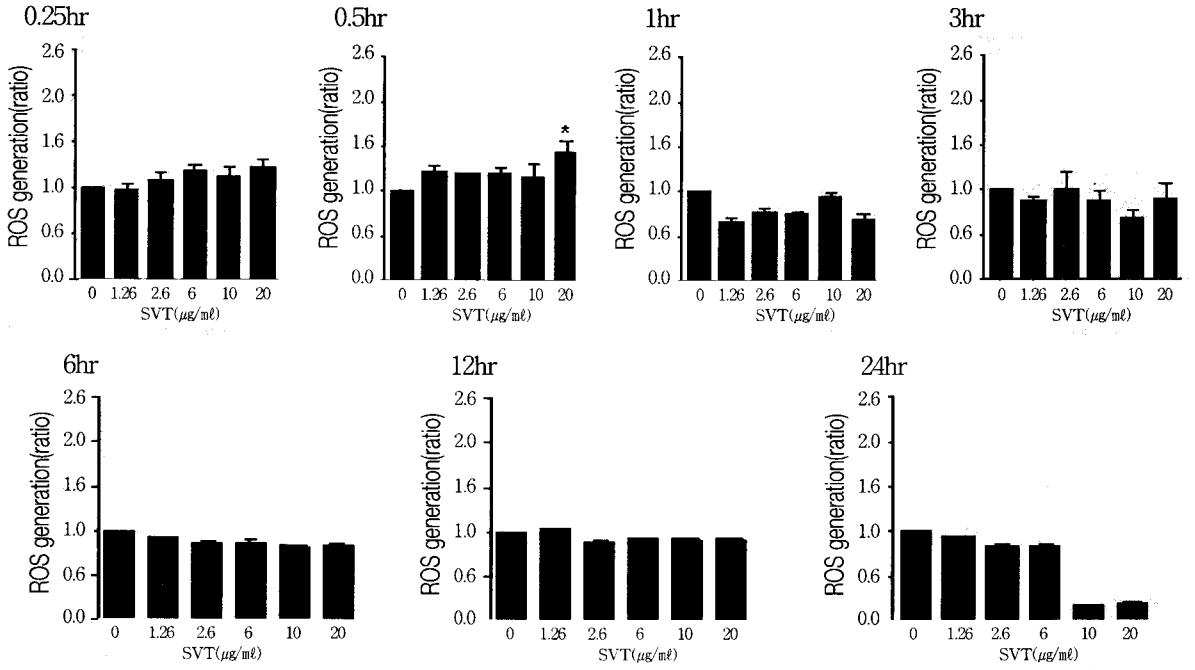

Fig. 5. ROS generation of MCF-7 cells by SVT according to a time course

ROS generation of MCF-7 cells was determined by ROS generation assay according to a time course as described under Method.

* represent P<0.05 significant compared with control.

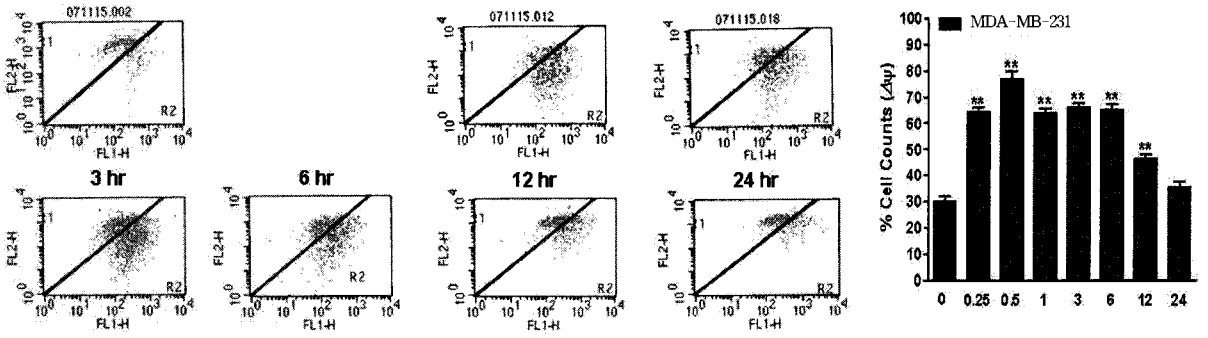

Fig. 6. Mitochondria membrane potential change of MDA-MB-231 cells by SVT according to a time course

Mitochondria Membrane Potential Change of MDA-MB-231 cells was determined by Mitochondria Membrane Potential measurement using JC-1 kit according to a time course as described under Method.

** represent P<0.01 significant compared with control respectively.

0.5hr(Fig. 5).

Mitochondrial membrane potential changed MDA-MB-231 cells were significantly increased at 0.25, 0.5, 1, 3, 6 and 12hr(Fig. 6), while MMP changed MCF-7 cells were significantly increased only at 0.25 and 0.5hr(Fig. 7).

MMP changed MDA-MB-231 cells significantly increased by 10μg/mℓ of SVT were 64.38±1.89, 76.98±3.12, 63.79±2.01, 66.01±1.56, 65.38±2.09 and 46.48±1.68 at 0.25, 0.5, 1, 3, 6 and 12hr respectively (Fig. 6), and MMP changed MCF-7 cells were 27.06±2.04 and 21.25±1.12 at 0.25 and 0.5hr(Fig. 7). MDA-MB-231 showed higher ROS generation level and change of MMP than MCF-7 presenting SVT possibly had more influence upon the mitochondria membrane potential of MDA-MB-231 and had the

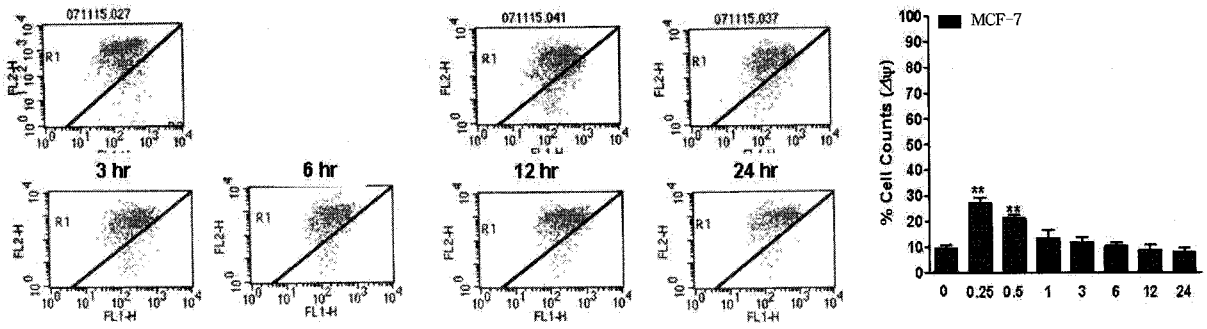

Fig. 7. Mitochondria membrane potential change of MCF-7 cells by SVT according to a time course

Mitochondria Membrane Potential Change of MCF-7 cells was determined by Mitochondria Membrane Potential measurement using JC-1 kit according to a time course as described under Method.
** represent P<0.01 significant compared with control respectively.

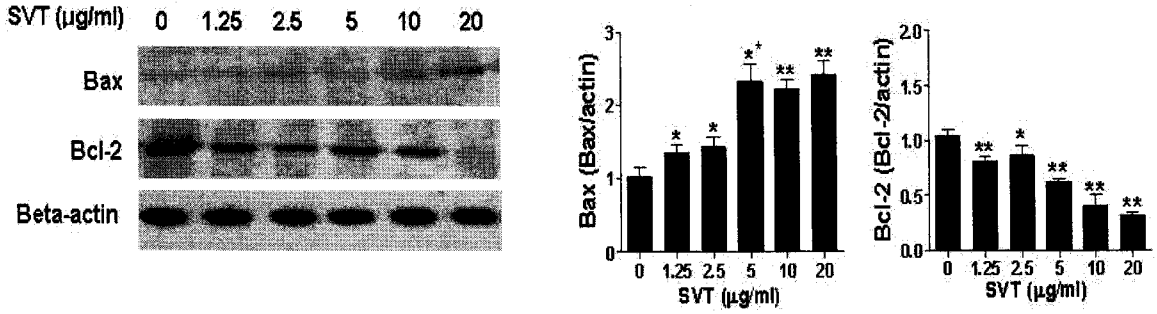

Fig. 8. Apoptosis related gene expressions of MCF-7 cells by SVT according to a time course

Bax, Bcl-2 were determined by western blot analysis at 24hr as described under Method.
* or ** represent P <0.05 or P<0.01 significant compared with control respectively.

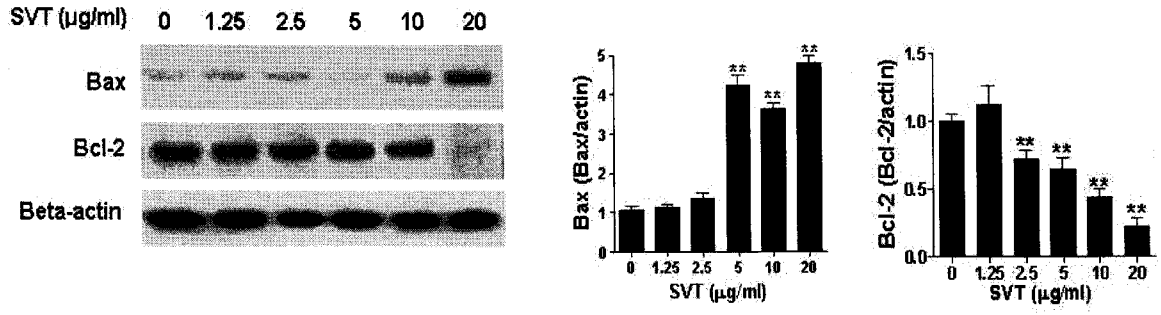

Fig. 9. Apoptosis related gene expressions of MDA-MB-231 cells by SVT according to a time course

Bax, Bcl-2 were determined by western blot analysis at 24hr as described under Method.
** represent P<0.01 significant compared with control respectively.

apoptosis undergo more vividly than MCF-7.

3. Expression of apoptosis regulatory proteins

Apoptosis is modulated by antiapoptotic and proapoptotic effectors, which involve a large number of proteins. Bcl-2 protein functions as a suppressor of apoptosis[15]. Bax is a proapoptotic protein and its predominance over Bcl-2 promotes apoptosis. Translocation of Bax protein causes change in mitochondrial transmembrane potential that plays

an important role in induction of apoptosis.

Compared with control, Bax in MCF-7 or MDA-MB-231 cells treated by 1.25, 2.5, 5, 10 and 20㎍/㎖ of SVT was increased in a dose dependent manner, whereas Bcl-2 in them were decreased dose dependently(Fig. 8, 9).

In MCF-7 cells, Bax significantly increased by 1.25, 2.5, 5, 10 and 20㎍/㎖ of SVT was 1.35±0.10, 1.42±0.14, 2.31±0.25, 2.21±0.13 and 2.40±0.20, and Bcl-2 significantly decreased by 1.25, 2.5, 5, 10 and 20㎍/㎖ of SVT was 0.81±0.05, 0.86±0.10, 0.62±0.03, 0.41±0.10 and 0.31±0.04(Fig. 8). In MDA-MB-231 cells, Bax significantly increased by 5, 10 and 20㎍/㎖ of SVT was 4.25±0.24, 3.65±0.15 and 4.81±0.18, and Bcl-2 significantly decreased by 2.5, 5, 10 and 20㎍/㎖ of SVT was 0.72±0.07, 0.65±0.08, 0.43±0.06 and 0.22±0.07(Fig. 9).

Ⅳ. Discussion

The central and novel finding in the present study is the identification of anticancer efficacy of SVT from *Vipera lebetina turanica* against human breast cancer MCF-7 or MDA-MB-231 cells. Most of the present available cytotoxic anticancer drugs mediate their effect via induction of apoptosis in cancer cells[16,17], and induction of apoptosis is suggested as one of the major mechanisms for the targeted therapy of various cancers including breast cancer[18,19]. Breast cancer is one of the most common human malignancies and the second leading cause of cancer-related deaths in women, with which about 30%−40% of women will develop metastases and eventually die from their disease[5].

Recent progress in diagnosis and therapy has increased the survival of women in estrogen-dependent breast cancer. However, the treatment options available for estrogen-independent tumors are far from satisfactory, and consequently carry a poorer prognosis. The intricate details of breast cell growth control have not been completely elucidated. Major gaps still exist on the interplay between genetic and hormone related factors, and dietary influences, which collectively play a major role on the control of mammary cell growth[2,20-22]. Therefore, response of breast carcinoma to various antitumor agents is only modest, particularly those administered as monotherapies[23]. Combination chemotherapies produce more clinically relevant, longer duration, better survival responses than single agents therapy and hence, have become the standard of management for metastatic, hormone-refractory breast carcinoma, and increasingly, also primary breast carcinoma[24]. These considerations provide the impetus for individuals diagnosed with breast cancer to explore prevention measures, including complementary and alternative medicine such as herbal medicine and pharmacopuncture for a more effective management and possibly cure of their disease.

Therefore, Developing the agents that induce apoptotic cell death of breast cancer cells could be a major step in controlling this malignancy. Elucidating important molecular mechanisms of specific toxin-receptor and/or ion channel complexes have been largely studied in drug discovery using natural toxins[25-27]. A snake venom toxin (SVT) from Vipera lebetina turanica, is a group of basic peptides, and important factor V activator composed of 236 amino acids with six disulfide bonds formed by twelve cysteins[13]. Shim et al.[14] reported that SVT induced apoptosis through significant increase of ROS generation and mitochondrial membrane potential change in neuroblastoma cells. On the basis of the insights gained from the above, I therefor conducted a mechanism study involving ROS generation, mitochondrial membrane potential and proapoptotic Bcl-2 family, determining whether SVT also exerts inhibitory effect on growth of human breast cancer cells including MCF-7 and MDA-MB-231, and how it works on the their apoptotic cell death.

At the very beginning, I investigated whether SVT can also inhibit the growth of breast cancer MCF-7 or MDA-MB-231 cells through CCK-8 according to time course.

SVT inhibited MCF-7 cell growth in a dose

dependent manner at 6 and 24hr, which was significantly decreased by 5,10 and 20㎍/㎖ of SVT at 6hr, by 1.5, 2.5 and 20㎍/㎖ of SVT at 12hr, and by 1.25, 2.5, 5, 10 and 20㎍/㎖ of SVT at 24hr. SVT also inhibited MDA-MB-231 cell proliferation dose dependently at 6, 12 and 24hr, which was significantly decreased by 1.25, 2.5, 5, 10 and 20㎍/㎖ of SVT at 6 to 24hr.

And then to delineate whether the inhibition of cell growth by the SVT was due to the induction of apoptosis, I evaluated apoptosis of MCF-7 or MDA-MB-231 cells by TUNEL assay and quantification using FACS. TUNEL-positive MCF-7 or MDA-MB-231 cells were increased dose dependently. MCF-7 cells demonstrated significant increase in the apoptosis rate induced by 10 and 20㎍/㎖ of SVT. MDA-MB-231 cells also showed significant increase of apoptosis rate in 2.5, 5, 10 and 20㎍/㎖ of SVT. Consistent with Shim[14]'s findings, current study demonstrated that SVT also inhibit human breast cancer MCF-7 or MDA-MB-231 cells proliferation through apoptosis.

Exocytotoxicity is a phenomenon in which cells undergo apoptosis[28], regarding the mechanism of which there are presently two schools of thought: the mitochondrial Ca^{2+} hypothesis and the nuclear poly-ADP-ribose polymerase (PARP) hypothesis, with both theories emphasizing a key role for ROS damaging cellular components such as proteins, lipids, and DNA[29-31]. Cells have endogenous buffering systems to circumvent the consequences of elevated calcium ions(Ca^{2+}) and reactive oxygen species (ROS) level[s32]. However, following injury to these buffering mechanisms or if the Ca^{2+} and ROS levels are too high for buffering systems, the cells succumb to their injury via multiple mechanisms[33-35]. Increased intracellular Ca^{2+} and ROS can activate DNA-damaging endonucleases, as well as introduce DNA damage directly[36-38]. In addition, increased intracellular Ca^{2+} can activate Ca^{2+}-dependent proteases including calpains; uncontrolled calpain activity damages many cytosolic proteins salient for cell survival, as well as damaging DNA[39]. Elevated Ca^{2+} and ROS can also affect mitochondrial function, thereby reducing ATP production, and, if uncontrolled, lead to mitochondrial permeability transition (MPT)[40,41]. MPT results in the release of intermitochondrial membrane proteins including cytochrome c, SMAC/DIABLO, endonuclease g, and apoptosis-inducing factor (AIF)[41,42].

These molecules, once released into the cytosol, mediate toxicity by introducing DNA damage and/or activating intracellular cascades leading to apoptosis[41,42]. The net effect of DNA damage, either via direct oxidation or by enzymatic cleavage, is the activation of energy-consuming DNA repair enzymes such as poly (ADP ribose) synthetase.

In the present study, moreover to elucidate a part of exocytotoxic celllular mechanism of apoptosis involved in the cells by SVT, I investigated the effect of ROS on the mitochondria measuring ROS generation level and confirming mitochondrial membrane potential changed MCF-7 or MDA-MB-231 cells. In MDA-MB-231 cells, SVT induced ROS generation was significantly increased in 10 and 20 ㎍/㎖ of SVT at 0.5hr, in 10㎍/㎖ of SVT at 1hr, and in 1.25, 2.5 and 10㎍/㎖ of SVT at 3hr, whereas MCF-7 cells did not have any significant change except that 20㎍/㎖ of SVT-induced ROS generation was significantly increased at 0.5hr.

Mitochondrial membrane potential changed MDA-MB-231 cells were significantly increased at 0.25, 0.5, 1, 3, 6 and 12hr, while MMP changed MCF-7 cells were significantly increased only at 0.25 and 0.5hr.

MMP changed MDA-MB-231 cells were significantly increased by 10㎍/㎖ of SVT at 0.25, 0.5, 1, 3, 6 and 12hr, and MMP changed MCF-7 cells were significantly increased at 0.25 and 0.5hr. MDA-MB-231 showed higher ROS generation level and change of MMP than MCF-7 presenting SVT possibly had more influence upon the mitochondria membrane potential of MDA-MB-231 and had the exocytotoxic mechanism- related apoptosis undergo more vividly than MCF-7.

Apoptosis is modulated by antiapoptotic and proapoptotic effectors, which involve a large number of proteins. Bcl-2 protein functions as a

suppressor of apoptosis[15]. Bax is a proapoptotic protein and its predominance over Bcl-2 promotes apoptosis. Translocation of Bax protein causes change in mitochondrial transmembrane potential that plays an important role in induction of apoptosis.

In this study, compared with control, Bax in MCF-7 or MDA-MB-231 cells treated by 1.25, 2.5, 5, 10 and 20㎍/㎖ of SVT was increased in a dose dependent manner, whereas Bcl-2 in them were decreased dose dependently.

In MCF-7 cells, Bax was significantly increased by 1.25, 2.5, 5, 10 and 20㎍/㎖ of SVT, while Bcl-2 was significantly decreased by 1.25, 2.5, 5, 10 and 20㎍/㎖ of SVT. In MDA-MB-231 cells, Bax was significantly increased by 5, 10 and 20㎍/㎖ of SVT whereas Bcl-2 significantly decreased by 2.5, 5, 10 and 20㎍/㎖ of SVT.

Consequently current study revealed that 1.25 to 20㎍/㎖ of SVT inhibited the growth of human breast cancer hormone sensitive MCF-7 or hormone refractory MDA-MB-231 cells through apoptosis via influence upon a part of exocytotic mechanism including ROS generation, mitochondrial membrane transition and cascading apoptosis related protein expressions such as Bax and Bcl-2 expression.

Although further study was needed to corroborate my findings, the present data suggested that it should serve to form a basis for the development of SVT as a novel agent for human breast cancer prevention and/or intervention.

V. Acknowledgement

This study was supported by a grant of Kyungwon University.

VI. References

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