Effect of Bee Venom Death Receptor Dependent Apoptosis and JAK2/STAT3 Pathway in the Ovarian Cancer

Ahn Byeong-joon and Song Ho-sueb

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

목적 : 이 연구는 봉독이 사람의 난소암 세포인 SKOV3와 PA-1에서 death receptor의 발현을 높여 세포자
멸사를 촉진함으로써 암세포의 성장을 억제하는지 밝히고자 하였다.

방법 : 난소암의 세포자멸사의 관찰에는 DAPI, TUNEL staining assay를 시행하였으며, 세포자멸사 조절
단백질의 변동 관찰에는 western blot analysis를 시행하였고, 난소암 세포에서 death receptor의 변화를 관찰
하기 위해 RT-PCR analysis를 시행하였다.

결과 : 1. DAPI, TUNEL staining assay 결과, 봉독은 투여량에 따라 세포자멸사의 유도를 통해 SKOV3와
PA-1 난소암세포의 증식을 억제하였고, 세포자멸사와 동반하여 DR4와 DR6의 발현이 두 암세포 모두에서 증
가하였고, DR3의 출현은 PA-1 세포에서 증가하였다.

2. Death Receptor의 발현 증가에 따라 caspase-3, 8, 9 and Bax를 포함하는 세포자멸사 촉진 단백질의 발
현이 동반하여 상승하였고 JAK2, STAT3의 인산화와 Bcl-2의 발현은 억제되었다.

3. siRNA 처리 시 봉독에 의한 DR3, DR4, DR6 발현증가와 STAT3의 활성억제가 역전되었다.

결론 : 이러한 결과는 봉독이 난소암 세포에서 DR3, DR4, DR6의 증가와 JAK2/STAT3 pathway의 억제를
동하여 세포자멸사를 유발한다는 것을 시사하며, 난소암의 예방과 치료에 효과적으로 활용될 수 있을 것으로

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* Corresponding author : Song Ho-sueb, Kyungwon Gil Oriental Medical Hospital, 1200-1
  Guwal-dong Namdong-gu Incheon Republic of Korea
  Tel. 82-70-7120-5012 E-mail : hssong70@kyungwon.ac.kr

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I. Introduction

Ovarian cancer is the most frequent cause of death from gynecological cancer\(^1\). Recent annual worldwide figures reflect 204,000 new cases of ovarian cancer and 125,000 deaths\(^2\). For the therapy of ovarian cancer, surgery is principle therapy\(^3\), and chemotherapy is also needed to remove the remaining cancer cells. However, the major challenge that limits the effectiveness of chemotherapy in patients with advanced ovarian cancer is the acquisition of resistance\(^4\). According to recent studies\(^5,6\), appropriate chemopreventive compounds reducing or overcoming resistance are believed to be a very hopeful strategy to reduce the incidence of ovarian cancer and enhance treatment efficacy\(^5,6\).

Apoptosis, the programmed cell death plays major role in anti-cancer effects of chemotherapeutics, which can be induced by activated caspase cascade systems through stimulation of death receptors via interaction of DRs with their ligands such as DR1 with TNF; DR2 with FasL; DR3, with Apo3L; DR4 and DR5 with TRAIL\(^7-14\).

Signal transducers and activators of transcription (STAT) proteins are transcription activators in JAK(specific inhibitors of Janus kinase)/STAT signal pathway, involving in cell growth, proliferation, survival, differentiation, apoptosis, metastasis, and angiogenesis\(^15\). Several studies have represented that phosphorylated STAT3 induce development and progression of various kinds of tumors such as breast, neck, head, prostate and ovary cancer, etc\(^16\). In other words, the inhibition of STAT3 by JAK can inhibit tumor cell growth and go apoptosis\(^17,18\).

Bee venom contains a variety of different peptides, including melittin, phospholipase A2, apamin, adolapin, and mast cell–degranulating peptide (MC DP). Bee venom has been used as a traditional medicine to treat back pain, rheumatism, and skin diseases by its antibacterial, antiviral, and anti-inflammatory effects\(^19\). Moreover, several studies have demonstrated that bee venom and/or melittin have anti-cancer effects including prostate, liver, breast, cervical, renal cancer cells\(^20-22\). However, experiments demonstrating the molecular mechanisms of the anti-cancer effects of bee venom in ovarian cancer cells have not been reported.

In this study, we therefore investigated anti-cancer effects of bee venom through increase of DR expression, but inhibition of STAT3 pathway in the human ovarian cancer cells, SKOV3 and PA-1.

II. Materials and methods

A. Materials

Bee venom was purchased from You-Miel Bee Venom Ltd. (Hwasoon, Jeonnam, Korea). The composition of the bee venom was as follows: 45-50% melittin, 25-3% mast cell degranulating peptide, 12% phospholipase A2, 1% lysophospholipase A, 1-1.5% histidine, 4-5% 6-pentyl a-pyrene lipids, 0.5% secarpin, 0.1% tertiapin, 0.1% procamine, 1.5-2% hyaluronidase, 2-3% amine, 4-5% carbohydrate, and 19-27% of others, including protease inhibitor, glucosidase, invertase, acid phosphomonoesterase, dopamine, norepinephrine, and unknown amino acids, with 99.5% purity. Caspase inhibitor (Z-VAD-FMK) was from Promega (Madison, WI). DR3, DR4, and DR6 siRNA were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA).