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Melittin and cancer

Anna Tacón

Department of Health and Exercise Science, Texas Tech University, Lubbock, USA

Address for correspondence: Anna Tacón, Department of Health and Exercise Science, Texas Tech University, Lubbock, USA. Fax: +806 742-1688. E-mail: anna.tacon@ttu.edu

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ABSTRACT

Cancer no longer is the automatic death sentence of centuries past, yet it remains a leading cause of global morbidity and mortality despite advances in detection, treatment, and survival in the field of oncology. The search for natural chemotherapeutic substances has accelerated research into the use of venom as a potential weapon against cancer. A short review of bee venom, in particular, melittin research, and its potential role in future cancer treatment is presented.

KEY WORDS: Bee venom, bee venom therapy, cancer, nanobees, oncology

INTRODUCTION

According to the World Health Organization, cancer remains a leading cause of global morbidity and mortality with approximately 14 million new cases and 8.2 million cancer-related deaths reported in 2012 [1]. Contemporary therapy usually involves some variation or combination of surgery, radiation or chemotherapy, each typically with its own form of discomfort and side effects. Since the incidence is expected to increase by 70% over the next two decades, the search continues for potentially new and effective chemotherapy agents with less of the well-known side effects commonly associated with chemotherapy [1]. Since 14 B.C., the venom of bees has been used to treat a variety of ailments and conditions including the treatment of pain and chronic inflammatory diseases such as rheumatism, multiple sclerosis, and skin diseases due to its antibacterial, nonsteroidal anti-inflammatory, as well as antiviral agents [2-6]. Bee venom (BV) is explored here in a short narrative review as a promising therapeutic agent for cancer.

NATURE OF THE PROBLEM

On a very simplistic and basic level, mistakes occur at the cellular level and accumulate in the development of this disease. The DNA repair and tumor suppressor genes malfunction as well as an error occurs in that the growth gene, which accelerates the division and production of rogue cells: The process of angiogenesis occurs to feed and supply blood to increasing growth of the malignant neoplasm; and when a healthy cell goes "AWOL" the new malignant cell's membrane transforms from its normal asymmetry to that of a malignant cell with a changed cell surface [2]. This is because the architectural structure of the cell membrane is altered due to changes in membrane components along with altered cellular biological properties and biosynthesis processes that occur. Indeed, changes in the levels of phospholipids and structural proteins at the cell surface have been hypothesized to reflect the degree of disintegration and impairment of genomic functioning that occurred as a result of mutations associated with the initial malignant rogue cell transformation [7].

The field of oncology has advanced modern treatments for cancer with a variety of strategies to treat a disease that centuries ago meant certain death; for example, disrupting cell division of cancer cells, damaging DNA, or preventing replication of DNA within the malignant cells [2]. Unfortunately, the toxic and imprecise targeting nature of potential chemotherapies, including BV therapy (BVT), can apply to all cells, which means that healthy cells also are destroyed in the process [3-8]. In the past decade alone, several toxins in nature have been found to have anticancer properties. That is, isolated molecules within venom compounds appear to damage and/or kill cancer cells, and one venom of recent biotechnological research as a new weapon for oncology is that of BV or BVT.

BV PROPERTIES

BV comes from the venom gland in the abdominal cavity of the bee and contains a variety of biologically active peptides that include of adolapin, apamin, melittin, and mast cell degranulating peptide as well as phospholipase A2 and hyaluronidase enzymes. Among these compounds, melittin, a small linear peptide consisting of 26 amino acids – isolated from honeybee *Apis mellifera* – is BV's major protein component, and constitutes, at minimum, 50% of the dry matter of BV [3,4,6-8]. Of biochemical import here is that melittin is the primary toxin

in BV, and simply put, the majority anti-tumor effect of BV is attributed to melittin [9]. Patients cannot simply be injected with venom, for there are harmful side effects. A bee sting is painful and gets inflamed precisely because melittin destroys cell membranes; other side effects may include blood clotting as well as damaging healthy nerve cells [2]. Yet, during the last two decades, melittin has attracted considerable research attention for its chemotherapeutic potential.

MELITTIN GENERAL PROPERTIES

This peptide with powerful hemolytic activity has the chemical formula $C_{131}H_{228}N_{38}O_{32}$, which weighs 2847.5 Da and contains the amino acid sequence of Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln [9]. Melittin, a natural detergent, is water soluble and disrupts natural as well as synthetic membranes; specifically, it forms tetramer aggregates as pores for ions, which produces disorder in phospholipid bilayers' structure [9]. This major substance of BV is tetrameric at concentrations in the bee's abdominal sack, yet it is monomeric at the minimum concentration necessary for the action of cell lysis. One considered benefit of this agent is its known attribute of initiating a variety of membrane-perturbing effects including hemolytic and antimicrobial activity; it induces pore formation, fusion, and vesiculation membrane alterations [10]. Melittin stimulates certain enzymes, for example, G-protein, protein kinase, adenylate cyclase, phospholipase C and phospholipase D, and moreover, plays a role in signal transduction. Finally, melittin is the first metabostatic peptide to inhibit the intrinsic activity of G protein [8].

MELITTIN APPLICATION PROPERTIES

Increasing research on BV as a weapon of nature has focused on the application of melittin to oncology due to various cancer application properties. For example, a basic property of melittin is that it is a nonspecific cytolytic peptide which attacks (all) lipid membranes and leads to significant toxicity when injected intravenously [8]. Anticancer activities of melittin have been found in breast, liver, leukemia, lung, mammary, and prostate, cancer cells [8,10-12].

Specifically, studies indicate that cytotoxic effects on cancer cells occur via activation of phospholipases A2, caspase, and matrix metalloproteinase-2, which result in the destruction of cancer cells [8,10-12]. Melittin specifically destroys cells that express the oncoprotein. Indeed, melittin reverts the transformed phenotype of H-ras transformed cells; and in culture, melittin selects cells exhibiting high levels of the ras oncogene [8,13].

Melittin appears to be one of the most potent inhibitors of calmodulin (CaM) properties that inhibit growth and clonogenicity of leukemia cells in humans [14]. Specifically, CaM is a ubiquitous Ca^{2+} receptor protein that mediates many signaling processes in eukaryotic cells; as well, CaM plays a central role in regulating a vast array of cellular functions via interaction with multiple target proteins [14]. CaM is of major import to cancer because Ca^{2+} mobilization is changed in cancer cells, which has implications for tumor growth and proliferation. Research has shown also that Ca^{2+} can communicate signals that induce cell death, that is, necrosis and apoptosis (programmed cell death [PCD]) also known as PCD. BV properties have induced apoptosis in cancer cells both *in vitro* and *in vivo* [8]. Apoptosis induced by melittin has been documented in gastric, lung, hepatocellular, ovarian, and renal malignant cells [4,15-18].

Another cancer application involves melittin's antimetastatic and antigrowth properties [19,20]. Research by Liu *et al.* demonstrated melittin's ability to inhibit malignant cell metastasis via reducing cell motility and migration – by suppressing the Rac1-dependent pathway [19]. The invasion and metastasis of rogue malignant cells are the primary reasons for cancer progression, and the process of angiogenesis is known for its essential role in such progression, which relies on migration, vascular cell proliferation, and endothelial tube formation [21,22]. Several studies have found that BV inhibits angiogenesis. For example, one study compared the antitumor effects of melittin and NS398, a cyclooxygenase-2 inhibitor, *in vivo* and *in vitro* [23]. Results showed that melittin produced more significant effects than NS398. Specifically, subcutaneous injections of melittin at 0.5 and 5 mg/kg significantly suppressed vascular endothelial growth factor (VEGF)-A-transfected highly metastatic Lewis lung cancer (VEGF-A-hm) tumor growth with a significant reduction in vessel number, respectively, by 25% and 57% [23]. Huh *et al.* concluded that melittin's mechanism may be anti-angiogenic actions of inhibiting VEGF receptor-2 and inflammatory mediators [23]. In addition, it has been found that anti-angiogenic activity of BV or melittin appears to occur during different stages of tumor progression and that melittin inhibits the growth and angiogenesis of liver cancer cells [21-24].

In sum, the following can be adduced regarding the mechanisms of cancer and melittin properties. As stated previously, when a healthy cell goes "AWOL," the new malignant cell's membrane transforms from its normal asymmetry to that of a malignant cell with a changed cell surface [2]. Architectural changes at the cancer cell surface appear to reflect disintegration and impairment of genomic functioning [7], which sets up specific instability and vulnerability. First, such disintegration and impairment of genomic functioning intuitively enhances melittin's property of being a cytolytic peptide that attacks and disrupts membranes and cellular communication; in addition, recall that melittin specifically destroys cells that express the oncoprotein, and even reverts the transformed phenotype of H-ras transformed cells [8,13]. Next, CaM is a ubiquitous Ca^{2+} receptor protein that mediates many signaling processes, plays a central role in essential cellular functions, and is significant to cancer because Ca^{2+} mobilization has implications for tumor growth and proliferation. Specifically, this Ca^{2+} receptor protein can communicate signals to induce cell death or apoptosis (PCD) [14]. Melittin is salient because it is one of the most potent inhibitors of CaM properties known which consequently affects growth and proliferation of certain

cancer cells. In addition to altering malignant growth and proliferation via CaM, melittin also can inhibit malignant cell metastasis by suppressing the Rac1-dependent pathway that is of import to tumor spread [19]. Finally, in terms of malignant progression, melittin has anti-angiogenesis properties [21-24]; that is; melittin inhibits the essential sustenance mechanism of angiogenesis in the growth or proliferation of cancer cells that give rise to tumor spread and invasion.

THE FUTURE NANOBEES AND SYNERGISTIC DEVELOPMENTS

In conclusion, one basic benefit of melittin simply may be that combining melittin with a known chemotherapeutic drug may be synergistic so as to reduce therapeutic doses, thus decreasing the total amount of toxins needed to destroy cancer [9]. Recall that melittin's anticancer potential has been known for decades, yet its major adverse effect of nonspecific cytotocity to both cancer and healthy cells, or lack of precision targeting, has made it prohibitive. Recent research, however, is enhancing target efficiency of cancer cells through the combination of BVT and nanotechnology. Specifically, nanoparticles serve as the delivery mechanism of melittin, while still preserving the integrity of healthy cells.

Soman *et al.* proposed the new paradigm for targeted delivery of melittin (and other classes of cell-penetrating peptides) via nanovehicles to kill cancer cells in mice *in vitro* and *in vivo*. Results demonstrated for the first time the ability to safely transport significant amounts of melittin intravenously, which destroyed the following types of experimental tumors: Syngeneic (B16F10 mouse melanoma); xenograft (MDA-MB-435 human breast cancer); precancerous lesions in K14-HPV16 mice with squamous dysplasia and carcinoma [25]. In sum, attaching melittin cargo to synthetically manufactured nanosized spheres results in a chemotherapeutic weapon transporter, referred to as nanobees that are injected into the blood stream to circulate in search of cancer cells [25-27]. Synthetic melittin has been developed in Pan's lab to reduce all potential side effects of venom for use with nanobee vehicles [28,29].

While anti-carcinogenic properties have been found for several bee products such as honey, pollen, and royal jelly; recent research activity has increased in developing (1) New venom/melittin-fused toxins and (2) chemotherapy combination testing. Specifically, a novel fused toxin, the disintegrin linker mellitin (DLM) linker, composed of melittin and disintegrin, urokinase-type plasminogen activator (uPA) have been developed; the linker, uPA, cleavable to tumor cells, allows DLM to release melittin [30]. Another recent development is the new fusion type protein of amino-terminal fragment (ATF)-melittin, a combination of melittin with the ATF of uPA, produced to target cancer cells [31]. The combination of BV with chemotherapy drugs also has been advanced this year, for example, the fused protein combination developed with melittin (MIL-2) and interleukin 2, a cancer therapy drug, as a promising candidate for cancer immunotherapy [32,33]. A similar investigation of combination involved the well-known drug, Cisplatin (cDDP) and BV. The synergistic effect of both agents, allowing a decrease in cDDP dosage, suggested a greater anticancer effect due to decrease in both side effects as well as potential development of cDDP resistance over the course of treatment [34]. Finally, the anticancer and imunomodulatory properties of propolis, produced by the honeybee that contain schrysin, have been known for several years, yet recent findings from BV and propolis combined treatments on breast adenocarcinoma and triple negative breast cancer cells show potential for future chemotherapy development [35-38].

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