Review Article :

Anticancer effects of melittin and composition of bee venom: Review article

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Abstract:

Apitherapy may be defined as the application of honeybee products, including honey, propolis, royal jelly, pollen, beeswax and especially bee venom, for medicinal and therapeutic purposes. Bee venom is commonly employed in the treatment of a number of immune-related diseases, and has recently also been used against tumors. Bee venom peptides including melittin and phospholipase A2 are capable of targeting leukemia, renal, pulmonary, hepatic, prostate, bladder, and breast cancer cells. Further research into bee venom therapy is now needed to identify specific components and target actions.

Key words: Bee venom, melittin, cancer

Introduction

Apitherapy may be defined as the application of honeybee products, including honey, propolis, royal jelly, pollen, beeswax, and bee venom for medicinal ends.[1] Bee venom has traditionally been used to treat a number of diseases since the very earliest times .[1] The therapy relies on a broad spectrum of pharmacologically active molecules contained in these crude extracts. This totality of chemical compounds consists of biogenic amine, enzymes (phospholipase A2), basic peptides and proteins (melittin and apamin) and various water-soluble and nitrogen-containing substances.[2] Peptides identified in BV include melittin, phospholipase A2, apamin, adolapin, and mast cell-degranulating peptide (MCDP).[3] Melittin, a small linear peptide made up of 26 amino acids, is the venom's major component.[4] Melittin acts as a natural detergent, exhibiting significant surface and membrane tension. It has also been described as a promising compound in the context of chemotherapy. Bee venom and/or melittin have also been shown to have anti-cancer properties including prostate, liver, breast, cervical, and renal cancer cells.[5] Melittin reacts with many metabolic cell functions, as well as disrupting the plasma membrane, resulting in modifications to the enzymatic system.[6] The purpose of this paper is to review recent studies of melittin and its therapeutic benefits.

Properties and Composition of Bee Venom:

Honeybee venom is a transparent fluid with a bitter taste that dries quickly at room temperature. The venom consists of a hydrolytic combinations of proteins with basic pH (4.5 to 5.5) and is used by bees for defense. Bee venom causes severe burning and irritation on contact with the mucous membranes or the eyes. The venom is soluble in water, but not in alcohol or ammonium sulfate. In contact with air, the venom forms pale gray crystals. Dried venom is light yellow in color, while some commercial forms are brown. This color change has been attributed to oxidation of various venom proteins. Bee venom contains several highly volatile compounds, which are susceptible to being lost during the collection process. It is widely regarded as a rich source of enzymes,

peptides and biogenic amines. Its specific weight is 1.1331,[1] and it consists of 88% water. Levels of glucose, fructose and phospholipid in venom are comparable to those in the animal's blood.[7] Bee venom is a complex combination of proteins, peptides and low molecular components, which have now been successfully characterized. The main difference between the compositions of fresh and dried venom lies in the volatile components, although the overall biological activities of the two forms are similar.[7,8]

Polypeptides:

Bee venom contains a large number of peptides, including melittin, apamin, adolapin, and MCDP, enzymes (phospholipase A2) biologically active amines (such as histamine and epinephrine), and various non-peptide elements with pharmaceutical properties.[9] Polypeptides have a lower molecular weight than enzymes, and consist of two or more amino acids. Melittin is the principal polypeptide and component of bee venom. Melittin has a molecular weight of 2840 Da, although this can be as high as 12,500 Da because melittin can also appear in a tetrameri form.[7] Melittin exhibits potent anti-inflammatory properties. However, at low concentrations it mediates mast cell degranulation and histamine secretion from mast cells, found in blood and all tissues perfused by it.[10,11] Bradykinin is a physiologically active peptide from the kinin protein group. Bradykinin and related kinins act on two receptors, known as B1 and B2. Expression of B1 occurs only as a result of tissue injury, and this receptor is implicated in chronic pain. B2 is expressed in a constitutive manner, and is involved in vasodilatation through the release of prostacyclin, nitric oxide, and endothelium-derived hyperpolarizing factor, resulting in a decrease in blood pressure.[12] Adolapin was first isolated from bee venom in the 1980s. It exhibits powerful analgesic and anti-inflammatory effects in rats, suppressing prostaglandin.[11]

Enzymes:

In the context of toxicity, phospholipase and hyaluronidase are the two major enzymatic proteins found in hymenoptera venom. These are capable of triggering an immune response, leading to IgE reactions in susceptible subjects.[13] Phospholipase A2 (PLA2) is a calcium-dependent enzyme responsible for hydrolyzing the sn-2 ester of glycerophospholipids, giving a fatty acid and a lysophospholipid. The enzyme eradicates phospholipids, compromising lipid bilayer integrity, meaning that cells become susceptible to additional degradation. PLA2 reaction products, including lysophosphatidylcholine, lysophosphatidic acid and sphingosine 1 phosphate, are capable of exhibiting cytotoxic or immunostimulatory effects on a range of different cell types, resulting in inflammation and immune responses.[14] Hyaluronidase has been described a "spreading factor" due to its hydrolyzation of the viscous polymer hyaluronic acid into non-viscous components. When hyaluronidase breaks down the extracellular matrix, the intracellular spaces facilitate infiltration by venom toxins. The venom thus penetrates tissues and passes into blood vessels, acting as a systemic poisoning catalyst. Additionally, hydrolyzed hyaluronan components exhibit pro-inflammatory, pro-angiogenic and immunostimulatory properties, thus accelerating systemic envenomation.[15]

Low molecular compounds:

Bee venom contains lower amounts of low molecular compounds that vary in in nature, including amino acids, physiologically active amines such as catecholamines, sugars and minerals.[7,8] Histamine is one of the principal components in this category. Histamine is an organic nitrogenous compound involved in the inflammatory response through enhancement of capillary permeability. Similarly, the catecholamines dopamine and nor-adrenaline both increase heart rate, thus facilitating both the circulation and distribution of the venom. As with histamine, however, the effects of dopamine and noradrenaline are to some extent masked by the activities of

other components of venom. Serotonin can act as an irritant and is capable of exacerbating venom-associated pain. Interestingly, high levels of acetylcholine have been determined only in wasp venom. Studies have reported that acetylcholine exacerbates sting-related discomfort by stimulating pain receptors in a synergic manner through histaminic effects.[11]

Melittin (Mel):

Melittin is a small linear basic peptide with a 26 amino acid sequence (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) and a molecular weight of 2847.5 kDa.[16] This peptide is an amphoteric molecule due to the specific arrangement of amino acids in the chain. Numerous effects have be reported, including antibacterial, antiviral, and anti-inflammatory activity in different cell types.[17]

Anticancer effects of melittin:

The inhibitory effect of melittin was first demonstrated in vitro by Hait et al. (1985).[18] They also reported that as a calmodulin inhibitor, melittin is capable of suppressing the growth and clonogenicity of human leukemia cells. Hait and Lee (1985) also observed inhibitory effects on astrocytoma cell growth, while Lazo et al. (1985) reported a similar effect in leukemia cells.[19,20] Melittin was also observed to enhance bleomycin toxicity in human granulocyte and macrophages and erythroid stem cell colonies.[21] Hait and Lee (1985) suggested that the cytotoxic activity of melittin was directly proportional to the antagonistic effect of calmodulin, as a potential intracellular target for the antiproliferative activity of melittin.[19] Studies have ascribed the cytotoxicity of melittin to both necrotic and apoptotic cell death. Melittin possesses the capacity to induce cell cycle arrest, growth inhibition, apoptosis and necrosis in a number of cancer cells. In vitro studies have shown that it creates significant growth inhibition and cytotoxicity against human hepatoma and glioma cell lines via apoptotic cell death.[22] Cytotoxic effects on cancer cells are mediated by the activation of PLA2, caspase, and matrix metalloproteinase-2, leading to the destruction of cancer cells.[23] Melittin specifically destroys cells responsible for oncoprotein expression. It reverts the transformed phenotype of H-ras transformed cells, while in culture, its selects those cells that exhibit elevated levels of the ras oncogene.[24]

Melittin is a particularly powerful inhibitor of calmodulin (CaM) properties which suppress the growth and clonogenicity of human leukemia cells.[25] Specifically, CaM is a ubiquitous Ca^{2+} receptor protein involved in numerous signaling processes in eukaryotic cells. In addition, it is crucially involved in regulating a very large number of cellular functions by means of interaction with numerous target proteins.[25] CaM is particularly important in the context of cancer due to the alteration in Ca^{2+} mobilization that occurs in cancer cells, with consequent implications for tumor growth and proliferation. Studies have also confirmed that Ca^{2+} is capable of communicate signals that induce necrosis and apoptosis, or programmed cell death (PCD). Bee venom components have been shown to induce apoptosis in cancer cells in vitro and also in vivo.[26] Such apoptosis has been reported in gastric, lung, hepatocellular, ovarian, and renal malignant cells.[27]

Another area of melittin application in the context of cancer concerns its antimetastatic and antigrowth properties.[5,28] Liu et al. confirmed that melittin is capable of inhibiting malignant cell metastasis by reducing cell motility and migration through inhibition of the Rac1-dependent pathway.[28] Invasion by and metastasis of rogue malignant cells are principally responsible for cancer progression, in which angiogenesis plays a fundamental role, based on migration, vascular cell proliferation, and endothelial tube formation.[29,30] Various authors have reported that bee venom inhibits angiogenesis. One study compared the antitumor effects of melittin and the

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cyclooxygenase-2 inhibitor NS398, both in vivo and in vitro.[29] The findings confirmed that melittin yielded more significant effects than NS398. In particular, subcutaneous injections of melittin at doses of 0.5 and 5 mg/kg significantly inhibited vascular endothelial growth factor (VEGF)-A-transfected highly metastatic Lewis lung cancer (VEGF-A-hm) tumor growth, with decreases in vessel numbers of 25% and 57%, respectively.[31] Huh et al. suggested that the effect mechanism of melittin may be associated with anti-angiogenic activity inhibiting VEGF receptor-2 and inflammatory mediators.[31]

Conclusion:

Mel is a promising candidate in the fight against cancer due to its broad-spectrum lytic properties.[32] Although melittin is cytotoxic to a wide range of tumor cells, it is also toxic to normal cells. A suitable means of delivery is therefore essential if melittin is to exhibit its full therapeutic potential. One possible solution to this might consist of melittin nanoparticles capable of safely delivering a significant amount of the compound via the intravenous route, to target and destroy tumors.[33,34] We suggest that detailed experimental studies investigating cellular and molecular mechanisms, accompanied by properly controlled, randomized clinical trials, may eventually result in the discovery of a therapeutic alternative in various disorders.

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