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Mini-review

Melittin, a major peptide component of bee venom, and its conjugates in cancer therapy

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ABSTRACT

Melittin (MEL), a major peptide component of bee venom, is an attractive candidate for cancer therapy. This agent has shown a variety of anti-cancer effects in preclinical cell culture and animal model systems. Despite a convincing efficacy data against variety of cancers, its applicability to humans has met with challenges due to several issues including its non-specific cytotoxicity, degradation and hemolytic activity. Several optimization approaches including utilization of nanoparticle based delivery of MEL have been utilized to circumvent the issues. Here, we summarize the current understanding of the anticancer effects of bee venom and MEL on different kinds of cancers. Further, we also present the available information for the possible mechanism of action of bee venom and/or MEL.

Introduction

Cancer is one of the major ailment effecting humankind and remains as one of the leading causes of mortality worldwide. The current available data suggests that over 10 million new patients are diagnosed with the disease every year and over 6 million deaths are associated with it representing roughly 12% of worldwide deaths. Fifteen million new cancer cases are anticipated to be diagnosed in the year 2020 [1] which will potentially increase to over 20 million by 2025 [2] and more in years to come. It is also anticipated that the growth and aging of the population may increase the new cancer cases to 21.7 million with about 13 million cancer deaths by the year 2030 [3].

Cancer development and progress are multifactorial process [4], either external factors such as tobacco, infectious organisms, environmental pollutants and an unhealthy diet or internal factors such as inherited genetic mutations, hormones, and immune conditions may act together or in concert to cause the onset of this disease [5]. Since cancer is associated with such high morbidity and mortality worldwide, there is an urgent need to determine ways of management of this ailment. The current treatment modalities are mainly comprised of surgery, radiation based therapy, chemotherapy, gene therapy and/or hormonal therapy [5,6]. All of these procedures utilized in mainstream medicine are almost always associated with significant unforeseen effects which pose challenge in its management. There has been an intense rush to devise alternative therapeutic approaches that have the potential for circumventing the usual side effects associated with mainstream medicines. We and others have suggested a concept of dietary intervention which has gained popularity and wide acceptance [7–12]. Another approach that has gained importance is the use of biotoxins such as animal venoms as cancer therapeutic agents [13–17]. These biotoxins are produced by living organisms as a defense mechanism against predators and are known to have both toxicological as well as pharmacological effects [18]. Current data suggests that toxin from bee venom (BV) has some potential as anti-tumor agent [19]. On the other hand, apitherapy, the medical uses of honey bee products range from royal jelly to BV, has been introduced as a natural therapeutics in cancer chemotherapy [20].

BV is a biotoxin or api-toxin synthesized and secreted by a gland that is present in the abdominal cavity of the bee and is composed of complex mixture of several biologically active peptides including Mellitin (MEL), enzymes, bioactive amines, and non-peptide components (Table 1) that has a variety of pharmaceutical properties [21]. Bee venom therapy (BVT), has been used in traditional medicine to treat diseases such as arthritis, rheumatism, pain, tumors, and skin diseases [22]. Studies have linked BV to variety of cancer

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Table 1
Dried bee venom composition.

Molecular group	Component	MW	Percent in dry venom
Enzymes	Phospholipase A2	19,000	10–12
	Phospholipase B		1
	Hyaluronidase	38,000	1.5–2
	Acid phosphomonoesterase	55,000	1
	α - Glucosidase	170,000	0.6
	Phosphatase		1
	Lysophospholipase		1
Peptides	Melittin	2840	40–50
	Apamine	2036	2–3
	MCD	2588	2–3
	Secapine	3000	0.5–2
	Pamine		1–3
	Minimine	6000	2–3
	Adolapine	11,500	1
	Procamine A, B	600	1.4
	Protease inhibitor	9000	<0.8
	Tertiapine	2500	0.1
	Cardiopep	2500	<0.7
	Melittin F		0.01
Phospholipids		700	1–3
Amines	Histamine	307.14	1.5
	Dopamine	189.64	0.13–1
	Noradrenalin	169.18	0.1–0.7
	Neurotransmitters		0.1–1
Amino acids	γ-aminobutyric acid	189.64	0.13–1
	α-amino acids	169.18	0.1–0.7
Carbohydrates	Glucose	180	2–4
	Fructose		
Pheromones	Iso-pentyl acetate; n-butyl acetate; iso-pentanol; n-hexyl acetate; n-octyl acetate; 2-nonanol; *n*-decyl acetate; benzyl acetate; benzyl alcohol; (2)-11-eicosen-1-ol	200	4–8

management effects including induction of apoptosis, necrosis, cytotoxicity and inhibition of proliferation in variety of cancer types of cancer cells, including prostate, breast, lung, liver and bladder [23]. Overall, BV and its selective components are considered promising agents for cancer management [19]. In addition, BV has also been linked with management of the side effects of cancer chemotherapy including a study where BV pharmacopuncture or MEL were used as a symptom-control therapy for chemotherapy-induced peripheral neuropathy [24]. However, the efficacy of BV appears to be due to the synergetic effect of MEL and this anti-cancer peptide might be the better choice than BV in native form [19].

MEL is the main active pharmacological component of BV, accounting for 40–50% of its total dry weight. It is a water-soluble, linear, cationic, hemolytic and amphipathic peptide weighting 2840 Da [25] and consisting of 26 amino acid (Fig. 1) with a chemical formula $C_{131}H_{229}N_{39}O_{31}$, the N-terminal region is mainly hydrophobic due to +4 charges while the C-terminal region is hydrophilic because of +2 charges hence the total is +6 charges at physiological pH [26].

Previous studies suggested the biological effects of MEL as antiviral, antibacterial, antifungal, anti-parasitic and anti-tumor and proposed the basis of MEL action as a non-selective cytolytic peptide which physically and chemically disrupts all prokaryotic and eukaryotic cell membranes [27–30]. MEL binds to negatively charged membrane surface (Fig. 2) and then disturbs the integrity of phospholipid bilayers by pore formation accompanied by the leakage of atomic ions and molecules and the enhancement of permeability that ultimately leads to cell lysis [31]. MEL was considered an attractive candidate for cancer chemotherapy causing more damage to the tumor cell membranes since its membrane potential is higher and cells are less likely to develop resistance to a membrane pore formation [32,33]. Although the potential applicability of MEL as a cancer chemotherapeutic agent has long been recognized, its rapid degradation in the blood and its nonspecific cellular lytic activity poses significant challenges [34]. MEL when injected intravenously causes severe toxic reactions such as hemolysis [35] which is a limiting factor for its widespread use for cancer therapy. Recently, it has been made clear that MEL and/or its conjugates can work in conjunction with hormone receptors [36], gene therapy [37] or as nanoparticles [33,34] for targeted therapies of some cancer types.

This review summarizes the current available literature about recent application of BV, MEL and different conjugates of MEL against several cancers both *in vitro* and *in vivo* (Table 2).

Anticancer effects of BV and its-conjugates

Effects on apoptosis

Apoptosis is an ordered and orchestrated cellular process that occurs in physiological and pathological conditions [38]. It is the main event that is known to regulate the occurrence and/or spread of cancer. Several studies have suggested that BV has potential anticancer effects against breast [39], hepatocellular carcinoma (HCC) [40], ovarian [41], prostate [42], melanoma [43], lung [44], leukemia [45] and cervical [46] cancers. It has been suggested that BV inhibits proliferation of the cancer cells via induction of apoptosis through multiple investigated mechanisms. In HCC, BV was shown to induce cytotoxic, genotoxic and mutagenic potential against HepG2 cells within three hours however it did not affect the mutagenicity induced by methyl methanesulfonate [47]. In another study, possible growth-inhibiting effects of BV applied alone or in combination with a cytotoxic drug bleomycin on HeLa and V79 cells was tested *in vitro*. Apoptosis, necrosis, and lysis were presumed as possible mechanisms by which BV inhibited growth and clonogenicity of V79 cells. HeLa cells, on the other hand, showed greater resistance to BV [46]. Another study investigated the mechanisms by which BV inhibits K1735M2 melanoma cells *in vitro* and B16 melanoma in C57BL/6 mice, *in-vivo* [48] Apoptosis was suggested as the possible mechanism by which BV inhibited cell proliferation and induced K1735M2 cell differentiation. The *in vivo* results showed that systemic administration of 1.0 and 3.0 mg/kg of BV resulted in significant inhibition of B16 melanoma growth with the relative tumor inhibition being 20 and 53% respectively. In another study, it was demonstrated that NCI-H1299 lung cancer cells treated with BV exhibit several features of apoptosis. In addition, expression of COX-2 mRNA and synthesis of PGE2 were inhibited by BV [49].

Choi et al. [44] reported in a study that BV induces apoptotic cell death in A549 and NCI-H460 lung cancer cells through the enhancement of death receptor 3 (DR3) expression and inhibition of NF-κB pathway. A combination treatment of TNF-like weak inducer of apoptosis, docetaxel and cisplatin, with BV synergistically inhibited both A549 and NCI-H460 lung cancer cell growth with further down regulation of NF-κB activity. In a parallel study, the authors used BV treated NK-92MI cells to co-culture with NSCLC cells and found that there is a further decrease in cell viability up to 70 and 75% in A549 and NCI-H460 cell lines respectively. Further, the DNA binding activity and luciferase activity of NF-κB was also inhibited after co-culture with BV treated NK-92MI cell lines. The knock down of death receptors with siRNA was observed to reverse the decrease in cell viability and NF-κB activity after co-culture with BV treated NK-92MI cells [50]. Similar effects of death receptor mediated BV activity was observed by Jo et al. [41], where they suggested that BV and MEL induces apoptotic