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Review Article
Exploration Through the Venoms from Hymenoptera as Potential Therapeutic Agents in Cancer Therapy

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Abstract

Side effects and drug resistance are important limitations to the currently available treatments against cancer. It is challenging therefore to search for new drugs that are effective antitumoral agents with slight or no side effects. There is a growing interest in natural products, among which Hymenoptera venoms show promising properties since they exhibit a cytotoxic activity on a variety of cancer cell lines. The objective of this contribution is to provide an updated state of the art on Hymenoptera venoms as potential anticancer agents. The present reviewed literature comprise the pharmacological activity on cancer models of Hymenoptera venoms, with a special attention to bees and wasps venom components. The crude bee venom and the peptides melittin and mastoparan are the most relevant products for various reasons, such as, their cytotoxicity on a variety of cancer cell lines, their diverse mechanisms of action and their ability to potentiate the anticancer activity of existing chemo agents and used clinically against cancer. Hence, Hymenoptera venoms appear as an important potential source for novel anti-cancer drugs, together with scorpions and snakes, the other animal groups that possess venoms with potent and varied pharmacological activities. *In vivo* studies are still needed to find the more relevant Hymenoptera candidates for clinical trials, opening the way for developing new, original drugs against cancer.

Key words: Hymenoptera venoms, cancer, bee venom, wasp venom, synergy bee venoms

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INTRODUCTION

Cancer is a leading cause of death worldwide with approximately 14 million new cases and 8.2 million deaths in the year 2012[1]. Besides to surgery and radiation therapy, chemotherapy is one of the most extensively used approaches for treating many cancers. However, the long-term use of chemotherapy can lead to drug resistance and severe side effects that are important limitations to the treatment effectiveness[2-3]. In recent years, there has been a growing interest for achieving more effective and rational cancer therapy, including the search for novel anticancer agents, especially among natural products[4].

Natural products have long been sources of relevant medicinal substances, with more than 70% of drugs on the market that are derived from or based upon natural compounds[5]. Many natural compounds have exhibited anti-carcinogenic activities by interfering with the initiation, development and progression of cancer through the modulation of various mechanisms including cellular proliferation, differentiation, apoptosis, angiogenesis and metastasis[6]. Several of these molecules have been tested in clinical trials and some of them gave promising results in combination therapy along with standard chemotherapeutic agents[7]. Most of the attention has been paid to phytochemicals, with several molecules that are among the most used anticancer drugs, such as vincristine and vinblastine, initially extracted from the Madagascar periwinkle *Catharanthus roseus* (Apocynaceae)[8], or taxol, originally isolated from the bark of *Taxus brevifolia* (Taxaceae)[9]. Several herbals are the most commonly used treatment in cancer patients worldwide[10]. Thereby, the most successful drugs from natural products have been isolated from plants, followed by microbes, fungi and marine organisms[11]. Surprisingly, in comparison, insects, which represent the largest and most diverse group of living organisms on Earth, with approximately 4,000,000 species, have received little attention despite the vast source of potential new drugs they represent[12]. Pharmacological studies revealed that natural products isolated from insects exhibit antibacterial[13], anti-inflammatory[14], antiviral[15], anti-cancer[16], anticoagulant and antiplatelet[17] effects, as well as properties on the central nervous system[18] among others. Especially, the order Hymenoptera stands out from the rest of the insects not only because of bee products such as honey, royal jelly, pollen, propolis and beeswax[19], but also because the venom extracts from bees and wasps have been found to exert promising properties against cancer and also against neurological diseases and allergies. For example, the venom-specific immunotherapy (VIT) is currently the only effective clinical treatment against insect venom allergy[20]. Recent reports revealed that the venom composition of wasps can greatly differ among closely related species, even when these species parasite the same host[21], as the case of the genus *Asobara*, whose species are endoparasitoids of *Drosophila* larvae[22]. Given the properties of bee products and the pharmacological potential as antitumoral agents of some venom compounds from bees and wasp species, it appeared timely to review the recent literature[4] to provide an overview of the state of knowledge about the compounds from Hymenoptera venoms as potential candidates for developing novel anticancer agents to be used alone or in combination with existing drugs. With this, it is expected to contribute to the scientific knowledge and also to the appreciation of the potential of venoms in certain types of cancer as future lines of research.

HYMENOPTERA VENOMS

The order Hymenoptera is one of the dominant insect groups on Earth, with more than 115,000 species, including bees, hornets, ants and wasps[23]. They show a high diversity of life styles, from socially organized to solitary species. Female wasps use stinging venom for prey capture and self-defense, which can cause local pain and damage and occasionally death, in large vertebrates, including man[24].

Venoms from hymenopteran are highly complex cocktails of bioactive compounds, which consist of a mixture of proteins (commonly referred to as toxins), salts and organic components, such as amino acids, alkaloids and neurotransmitters[25,26]. The chemical composition of the venoms is highly variable among species, as well as the abundance of some compounds, even in species from the same genus[27,28]. Nevertheless, proteins and peptides represent the main components in the venoms of bees and wasps and both contain several enzymes, such as phospholipases A2 and B, hyaluronidase and neurotransmitters such as serotonin, histamine, dopamine, noradrenaline and adrenaline. Beside, some peptides are exclusive to each insect[29]. Figure 1 shows a general structure of the present review, remarking the main Hymenoptera venom products with anticancer properties.

BEE VENOM

Honeybees' venom (*Apis mellifera*) has long been used in traditional medicine for the treatment of chronic inflammatory diseases. A number of studies have shown that

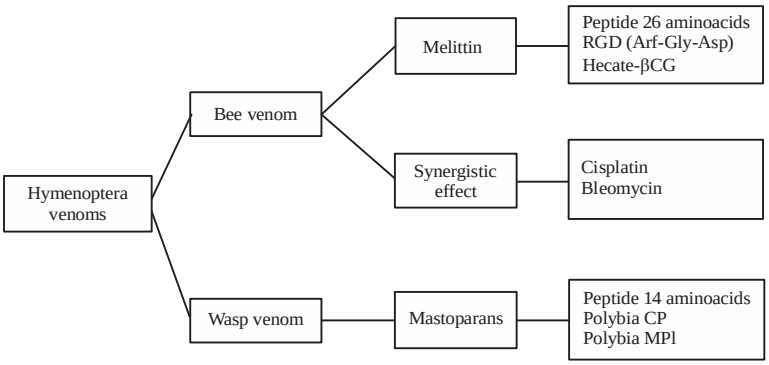

Fig. 1: Main Hymenoptera venom products with anticancer properties

bee venom exhibits a variety of physiological activities, such as antiarthritic, anti-inflammatory and anticancer actions[30].

The bee venom is a complex mixture of proteins and peptides, enzymes, amines, amino acids, sugars, phospholipids and volatile compounds. From these, the two major components are the peptide melittin, which represent approximately 50% of the dry venom and the enzyme phospholipase A2 (12% approximately)[31]. Recent reports[26,29] revealed different anticancer properties of the bee venom, where the inhibition of proliferation and induction of apoptosis are the most studied properties. For example, bee venom was able to inhibit the growth of SMMC-7721 human hepatoma cells by inducing apoptotic cell death via the reduction of the Ki67, a protein which is expressed in proliferating cells, while in *in vivo* experiments, the bee venom injected to the BALB/c nude mice was able to delay the tumor cell growth and the induction of apoptosis was confirmed by DNA fragmentation[32]. In another case, when human lung carcinoma cell line NCI-H1299 was treated with a 10 μg mL^{-1} dose of bee venom, notable changes were recorded in the morphology of these carcinoma cells, such as cytoplasmic blebbing, nuclear shrinkage, chromatin condensation, irregularity in shape and retraction of processes, all of which revealed apoptosis, which was confirmed by DAPI staining and DNA fragmentation. Moreover, bee venom was able to exhibit a selective inhibition of COX-2 mRNA expression, indicating a distinct anti-tumoral property, since COX-2 is an enzyme which is often up-regulated in tumors[33]. Other contributions evidenced the induction of apoptosis by bee venom on MCF7 breast cancer cells because of morphological changes, inhibition of proliferation, induction of the production of Reactive Oxygen Species (ROS) and dysfunction of the mitochondrial membrane potential. Then, apoptosis was induced by the release of cytochrome c that increased the levels of caspase-9 and poly (ADP-ribose) polymerase (PARP)[34].

Bee venom has also been considered a potential anti-metastatic and anti-invasive agent since it inhibits PMA-induced MMP-9 (matrix metalloproteinase-9) expression and activity by inactivating NF-κB via p38 MAPK and JNK signaling pathways in breast cancer cells MCF7[35]. Some *in vivo* experiments revealed that bee venom inhibits the proliferation of carcinoma cells (mammary carcinoma -MCa-) and tumor growth in CBA mouse by stimulation of the local cellular immune responses in lymph nodes when was injected intravenously or intratumorally, however, no effect was observed when the bee venom was administered subcutaneously[36].

Melittin: This amphiphilic peptide contains 26 amino acids and is the principal bioactive component derived from the venom of the bee *Apis mellifera*, representing 40-60% of the dry venom. Melittin has been recognized as the main toxin causing pain, inflammation and hypersensitivity[11].

Over the last decades, melittin has been the focus of a number of studies investigating its potential antitumoral activity against various cancer cells[37]. Melittin induces apoptosis of human leukemic cells U937 by activating caspases[38], with the up-regulation of Bax and caspase-3 activation and down-regulation of Bcl-2 and the inhibition of apoptosis IAP (inhibitor of apoptosis)[39]. Melittin has also been found to develop cytotoxic effects against malignant human glioma (U87 and U251 cells)[40], osteosarcoma and fetal osteoblasts[41], breast cancer (MCF7 cells)[34] and Vascular Smooth Muscle Cell (VSMC)[42]. However, these cytotoxic effects were observed in both tumor cells and normal cells, due the membrane-disrupting lytic properties of melittin, delaying the therapeutic development of melittin. Nevertheless, melittin had low toxicity when coupled with target peptides such as an immune conjugate of melittin, an adenovirus-melittin, a melittin-avidin conjugate and a RGD-melittin conjugate

(Arg-Gly-Asp) which has inhibitory effects in models of breast, ovarian and prostate cancers due its interaction with integrins on the surface of the tumor cells[43]. Other efforts have been considered melittin and a fragment of a melittin-conjugated hormone receptor as Hecate-βCG since the presence of lutropin/choriogonadotropin (LH/CG) receptors have been found in several cancer lines such as breast, ovarian and prostate. The conjugated consisted on the 23-amino acid lytic peptide Hecate and a 15-amino acid segment of β-chain of CG. Hecate-βCG has been shown to selectively kill cells expressing LH/CG receptors and that its toxicity depends on the number of binding sites for LH/CG per cell. Melittin-conjugated hormone receptors may thus be useful in cancer therapy due to its high toxicity and specificity for cancer cells[44].

The melittin/avidin conjugate exhibits a high cytolytic activity in DU 145 prostate cancer cells and SK-OV ovarian cancer cells that both exert high MMP-2 (matrix metalloproteinase-2) activity, but low cytotoxicity on normal cells with low MMP-2 activity. *In vivo*, the size of tumors injected with the melittin/avidin conjugate was significantly smaller as compared to untreated tumors[37,45]. Melittin conjugates showed specific and high toxicity for a variety of cancer cells: ovarian and testicular tumors, melanoma, hepatoma cells and prostate cancer cells[37,40]. Other conjugates (peptide 101) are designed based upon a portion of amino acids from melittin and a monoclonal antibody. This immunoconjugate exhibited strong inhibition of tumor growth when administered to mice bearing-tumor human prostate carcinoma xenografts[46]. Hence, we conclude that melittin-conjugates have a huge potential for the development of novel anticancer drugs.

On the other hand, melittin causes the death of several cell types by apoptosis, via the activation of phospholipase A2 or necrosis. Melittin is thus observed as a rather selective phospholipase A2 activator in Ras-transformed cells by the mediation of enhanced influx of calcium ions (Ca^{2+}) cells, suggesting a causal relationship between melittin and Ca^{2+} [47]. However, recent findings showed that melittin induced $[Ca^{2+}]i$ increases in MG63 osteosarcoma cells via Ca^{2+} influx through L-type Ca^{2+} channels and also causes apoptosis independently from the activation of phospholipase A2[48].

A different approach showed that melittin, when is combined with TNF-Related Apoptosis Inducing Ligand (TRAIL), it can induce the apoptosis of hepatocellular carcinoma (HCC) cells, potentially by activating CaMKII-TAK1-JNK/p38 signaling pathway while inhibiting IKK-NFκB pathway. This result provides a new area of study for HCC, one of the most aggressive malignant tumors which is highly prevalent around the world with still a very low survival rate. This combination may further be a promising therapeutic approach in the treatment of TRAIL-resistant human cancer[49].

WASP VENOM

The general composition of the wasp venom is also a proteinaceous cocktail of mainly peptides and enzymes. In some reports of the species from the parasitoid wasp *Asobara*, one of the most abundant venom proteins was identified to be an aspartylglucosaminidase[50,51]. Nonetheless, among the most relevant differences with the bee venom is the absence of melittin, apamin and Mast Cell Degranulating (MCD) peptide. However, other peptides, such as bradykinin and mastoparan were specifically identified in the wasp venom[29]. Hypotensive and anti-inflammatory activities have been reported for bradykinins[24], as well as neurotoxic properties[52]. Other major biological properties of the venoms from wasp are antimicrobial, inhibition of melanization and interruption of the development[26]. Cancer-related activities were described only for the mastoparan[29,53-55].

Mastoparan: The peptide mastoparan is composed by 14 amino-acids and is obtained from the venom of *Vespula lewisii*. Among other biological effects, it induces mitochondrial permeability transition which mediates its tumor cell cytotoxicity[29]. Recent studies showed that mastoparan induces apoptosis in B16F10-Nex2 melanoma cells through the intrinsic mitochondrial pathway, conferring protection against tumor development[53]. The antitumor effect of mastoparan was also tested in leukemia, (Jurkat and THP-1),myeloma (HOPC murine myeloma) and breast cancer cells (MDA-MB-231, T47D, MDA-MB-468, 4T1, SKBR3, MCF7 and paclitaxel-resistant MCF7-TX400)[53]. Mastoparan was toxic to all cell lines, including resistant strains, which is consistent with a mechanism that involves direct interactions with the cell membrane. As mastoparan is equally toxic to rapidly dividing and slow growing cells, it may be active toward indolent and slow-growing tumors, giving it an advantage over conventional chemotherapies, which target only rapidly dividing cells. Besides, mastoparan exhibited potential synergistic effect when tested in combination with gemcitabine, a drug that targets myeloid-derived suppressor cells. This combination significantly delayed tumor growth ($p<0.05$, in comparison to saline-treated mice) with differences being noted in both tumor volume and mass, suggesting that mastoparan has great potential in the treatment of various cancers[56].

Another mastoparan, originally isolated from the social wasp *Polybia paulista*, is the mastoparan polybia-CP, recognized as a natural product with antibacterial properties[54]. The mastoparan polybia-CP was recently synthesized and evaluated for its cytotoxic properties on tumor cells[55], showing potent antitumor activity against human bladder (Biu87) and prostate cancer (PC-3) by disrupting the integrity of the cell membrane. It was also found that polybia-CP adopts an amphipathic-helical conformation in the membrane, mimicking environment, while it takes an unordered conformation in water. In consequence, polybia-CP represents a potential novel therapeutic strategy in the treatment of tumor and infectious diseases, in the context of increasing multidrug resistance[55].

Polybia-MPI, another mastoparan which was also isolated from the venom of *Polybia paulista*, showed a significant inhibition (>90%) of the proliferation of the tumor cells (two human bladder cancer cell lines -Biu87 and EJ- and one prostate cancer cell line (PC-3-) by membrane disrupting, whereas the proliferation was relatively unaffected in non-tumorigenic cell line NIH3T3 (normal mouse fibroblast cell line). Then, polybia-MPI is relatively safe for cells unaffected by tumors, indicating cell selectivity. This dual effect of polybia-MPI on both proliferating endothelial cells and tumor cells likely enhances its antitumor activity[57].

SYNERGISTIC EFFECTS OF HYMENOPTERA VENOMS WITH ANTICANCER PRODUCTS

The interaction between biologically active agents having different targets is highly relevant since combinations usually allow reducing concentrations of the most toxic drug and thus decreasing the systemic toxicity[58]. In this context, there is a growing interest in combining natural products to chemotherapy[59]. Bee venom, as a natural toxin known for its cytotoxic effects towards a variety of cancer cells, which preferentially kills tumor cells than non-tumor cells, has been incorporated to several current anticancer treatment strategies in combination with standard chemotherapy[60].

Cisplatin (*cis*-diamminedichloroplatinum II), one of the most commonly used anticancer drugs, is very effective against a wide spectrum of solid neoplasms, including testicular, bladder, ovarian, colorectal, lung and head and neck cancers[61] but its use is often limited by severe side effects, including nephrotoxicity, neurotoxicity, ototoxicity, gastrointestinal disorders and hemorrhages[62-63]. Moreover, more and more acquired resistances to cisplatin treatment emerge, as a result of drug exposure[64], decreasing the clinical usefulness of cisplatin as an anticancer drug[61]. Therefore, some studies evaluated the possible joint anticancer effect of bee venom with cisplatin. A recent *in vitro* study demonstrates a synergistic effect of bee venom and cisplatin on human glioblastoma A1235 cells. In this study, a pre-treatment with bee venom increased the cytotoxicity of cisplatin towards A1235 cells, suggesting the possibility to reduce cisplatin concentrations during cancer treatment and postponing the development of cisplatin resistance[65].

Other authors that used MTT assays, found that 4 μg mL^{-1} bee venom/10 mg mL^{-1} cisplatin killed approximately 50% of A2780cp cells (human ovarian cancer cells) after 24 h. Additionally, the combination of bee venom with cisplatin induced an apoptotic type of A2780cp cell death, suggesting that bee venom has the potential to enhancing the cytotoxic effect of cisplatin[66].

The combination of bee venom with cisplatin was tested on human cervical (HeLa) and laryngeal carcinoma (HEp-2) cells and their drug resistant sub-lines, cisplatin-resistant cervical carcinoma cells (HeLa CK) and cisplatin-resistant laryngeal carcinoma cells (CK2). The synergy index (by isobologram analysis method) on HeLa and HeLa CK cell lines, at all concentrations tested indicated that a combination of bee venom and cisplatin could be synergistic although the synergistic effect was weak. Similarly, for the HEp-2 and CK2 cells, some bee venom and cisplatin concentrations gave only an additive effect. To sum up, a combined treatment with bee venom and cisplatin exhibits an additive and/or weakly synergistic effect towards the tested cell lines[60].

Bee venom has also been tested in combination with a novel palladium (II) complex Pd (II), [Pd(bpy)(Pi-Pydtc)]NO_3 against the human T-cell acute lymphoblastic leukemia cell line (MOLT-4). Concentrations of 1 μg mL^{-1} for bee venom and 0.85 μM for Pd (II) complex induce MOLT-4-cell apoptosis in a caspase-3-dependent manner[67]. In addition, the lethal effect of Pd complex was potentiated by adding a non-lethal dose of the bee venom, which is thought to synergistically act by preventing recovery from Pd complex-induced DNA damage[67].

On the other hand, bee venom has been also tested in combination with bleomycin, a chemotherapy drug used to treat testicular cancer, various lymphomas, cervical cancer and cancers of the head and neck[68]. This combination was tested on human cervical carcinoma cells (Hela) and Chinese hamster lung fibroblasts (V79 cells). In both cases, the lethal effect of bleomycin was potentiated by adding a non-lethal dose of the bee venom, causing a dose-dependent decrease in cell survival due to DNA damage. This suggests that bee venom might find a therapeutic use by enhancing cytotoxicity of antitumor agent bleomycin[69].

PERSPECTIVES FOR HYMENOPTERA VENOMS IN CANCER THERAPY

Cancer prevalence increases worldwide, due to the increasing demography, the aging of the population and the increasing prevalence of established risk factors such as smoking, overweight, physical inactivity and changing reproductive patterns associated with urbanization and economic development[70]. It is thus challenging to develop new drugs with effective anticancer properties. Nature is an excellent source of novel potential chemotherapeutic agents and provides compounds to chemistry for semi-synthesis or total synthesis[71]. With this respect, Hymenoptera venoms are a potential but still under explored source for new molecules that could be used clinically on their own or in combination with other drugs. Based on the literature reviewed in the present contribution, the venoms from bees and wasps emerge as the best candidates for the development of novel anticancer agents due the range of cancers against which they have proven cytotoxic activity in both *in vitro* and *in vivo* studies, because the diverse mechanisms by which they act, as well as their ability to potentiate the effect of chemo-agents when used in combination.

It is noteworthy that among the variety of cancers against which Hymenoptera venoms have a toxic effect, breast cancer is one of the most interesting to study in the future, since breast cancer in women is the most prevalent worldwide with 5.2 million cases and one in six cancer survivors diagnosed in 2008, additionally to the increasing frequency of drug resistance to all forms of systemic treatment[72]. In this context, bee venom and the peptides melittin and mastoparan, are the most relevant products reviewed in the present contribution that have been tested and showed anti breast cancer properties. It looks also interesting to may be test these bee venom products in Gonadotropin-Releasing Hormone (GnRH) and its receptor (GnRHR) since both are expressed by a number of malignant tumors, including those of the breast[73], providing opportunities for designing mechanism-based adjuvant therapies for breast cancer. Hence, the order Hymenoptera emerges as an important source of animal venoms that possess potential anti-cancer properties, after the scorpions and snakes. Since even only few compounds isolated from these later groups have been considered in clinical studies[74], the *in vitro* anti-cancer properties exerted by bee and wasp venoms still require further *in vivo* studies to reveal candidate molecules for clinical trials, opening the way to new chemo agents in cancer therapy.

CONCLUSION

Hymenoptera venoms exhibited *in vitro* anticancer properties in a wide variety of cancers and possess the capacity to potentiate the activity of chemo agents already used in cancer chemotherapy. Hence, Hymenoptera emerges as an important potentially source of anti-cancer animal venoms together with scorpions and snakes, the other animal groups that possess venoms with potent and varied pharmacological activities. Future Hymenoptera anticancer trials conducted in animals may be focused on crude bee venom and also melittin and mastoparan, due to their remarkable antitumoral activity.

SIGNIFICANCE STATEMENT

This work pretends to introduce the study of the natural sources like venom products as potential agents that may be used in the therapy of cancer in a future. This manuscript contents a general introduction about venoms of insects (Hymenoptera) and the most remarkable products derived from them. Hence, a scientific review of the literature related as well as the mechanisms of action of the crude extracts of venoms and their individual components is offered. Diverse types of cancer are impacted by these products but future experiments are necessary to define the *in vivo* effects of these venom products. Then, this work also could be an invitation to take another alternative natural group to study in the cancer research area.

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