Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 6, Issue 9, 2014

Review Article

ANIMAL VENOM FOR TREATING BREAST CANCER

SANCHITA AHLUWALIA*, NAKUL SHAH

Haffkine Institute of Training, Research & Testing Mailing address: 2001/2, Royale A Wing, Neelkanth Palms, Off Ghodbunder Road, Thane, Mumbai, Maharaashtra, India 400610
Email: sanchita.ahluwalia@gmail.com

Received: 04 Aug 2014 Revised and Accepted: 13 Aug 2014

ABSTRACT

Breast cancer is one of the most common malignancies found in women and is associated with increased mortality in advanced disease. With the use of improved screening techniques and improvisation in treatment, 89% of the women diagnosed with breast cancer will survive 5 years from diagnosis. Despite significant advancement in the diagnosis and treatment of breast cancer, many patients succumb to this disease. The elucidation of aberrant signaling pathways that lead to breast cancer should help develop more effective therapeutic strategies. This review focuses on the different targets of breast cancer and how they can be acted upon by different animal venoms.

Keywords: Breast, Cancer, Target, Venom.

INTRODUCTION

Female breast cancer is the most prevalent neoplasm worldwide with 5.2 million cases, and one in six cancer survivors in 2008 diagnosed within the previous 5 years [1].

Current treatment options include surgery, radiation and systemic therapy (e. g. Hormonal therapy, chemotherapy and biological therapy). Toxic side effects and emergence of drug resistance are the major problems faced with chemotherapy. The development of rational therapeutic approaches for breast cancer requires an understanding of the cellular signaling pathways leading to breast cancer. The development of successful therapeutic agents requires not only identification of appropriate molecular targets but, also elucidation of the signaling pathways that may contribute to the resistance to these agents.

Targeting and characterizing the regulation of different pathways involved in anticancer can prove helpful in defining new therapeutic targets to develop chemotherapies with fewer side effects and toxicity, without compromising their therapeutic efficacy for treating cancer.

The researchers have found a way, using the burgeoning field of nanotechnology, to pinpoint tumours for attack by melittin while largely shielding healthy cells. The resultant products, called nanobees, are injected into the blood stream, where they circulate until they reach and attack cancerous tumours. The approach also has the potential to avoid some of the toxic side effects seen in older cancer therapies like chemotherapy [2, 3].

It has been shown that the snake venom toxin inhibits growth of colon cancer cells through induction of apoptosis [4]. Snake venom toxin from *Vipera lebetina turanica* causes apoptotic cell death of ovarian cancer cells through the inhibition of NF-κB and STAT3 signal, which suggests that the snake venom toxin may be applicable as an anticancer agent for ovarian cancer [5]. A study used Cobra venom cytotoxin (CVC) loaded in poly (lactide-co-glycolide) (PLGA) microspheres mixed with ricin and encapsulated in a thermosensitive PLGA-PEG-PLGA hydrogel.

The *in vivo* experiments shows that intra tumoral injection of SSRP could inhibit hepatocellular carcinoma growth significantly, and the tumour growth inhibition rate reached 73.5% [6]. Crotoxin (CrTX), a potent neurotoxin extracted from the venom of the pit viper *Crotalus durissus terrificus*, produces significant anti-tumour effects on A549 cell line by inducing cell apoptosis probably due to activation of P38MAPK and caspase-3, and by cell cycle arrest mediated by increased p53 expression. In addition, CrTX displays anti-angiogenic effects *in vivo* [7].

Cure for thousands of cases of cancer has emerged from the blue scorpion venom, endemic to Cuba. The blue scorpion venom contains a protein chain that attacks cancer. The "escozul" also acts as "immuno-modulator," raising the defences [8]. A treatment based on scorpion venom loaded with radioactive material is being tested as a way to kill brain tumour. A protein in venom from the yellow Israeli scorpion has been found to bind preferentially to the glioma cells, so scientists have created a synthetic version that does not by itself kill a patient. When the venom protein attaches to the glioma cells, the radiation kills them. Health physicists in a study used a compound called TM-601, a synthetic version of the molecule. The molecule, a protein, was bound to a radioactive substance called I-131 believed to kill glioma cells [9].

Lewis tumour model has been established to explore the mechanism of polypeptide extract from scorpion venom (PESV) on promoting anti-tumour effects of cyclophosphamide (CTX). In this model, the extract was subcutaneously implanted into Lewis lung cells into C57BL/6 mice. Inhibition of expressions of VEGF and TGF-beta1, promotion of maturation of dendritic cells, recovery of antigen uptake presentation function, and reversal of immune injury to the body by CTX was seen with PESV, thus playing a role in inducing the tumour cell apoptosis [10].

Bufalin from the skin and parotid venom glands of toad has shown to induce apoptosis cell death in many types of cancer cell lines. It might be used as a therapeutic agent for the treatment of human bladder cancer [11]. Cinobufacini (Huachansu), an aqueous extract from the skins of *Bufo bufo gargarizans* Cantor, is a well-known traditional Chinese medicine widely used in clinical cancer therapy in China. These data suggested cinobufacini could induce apoptosis of hepatocellular carcinoma cells through mitochondria and FAS-mediated caspase-dependent pathways with an increase of treatment time, which might provide an experimental evidence for cinobufacini treatment in human hepatocellular carcinoma [12].

Evaluation of the anti-tumour potential of samsum ant venom (SAV) from *Pachycondyla sennaarensis* on the human breast carcinoma cell line MCF-7 has been done. It was found that SAV induces growth arrest of MCF-7 cells without affecting the viability of MCF-10 (non-tumorigenic normal breast epithelial cells) and normal PBMCs [13].

This review provides a brief discussion on selected targets for breast cancer.

Bee Venom

Bee venom contains several physiologically active components. Two of the most toxic compounds are melittin and phospholipase A2

(PLA2). Melittin constitutes 30% to 50% of dry bee venom, and PLA2 makes up about 10% to 12% of bee venom. Melittin is a potent hemolytic that causes mast cell degranulation and activates PLA2. Other constituents in bee venom include hyaluronidase, apamin, mast cell degranulating peptide (MCD peptide), procamine, secapin, tertiapin, and other small peptides including adolapin along with histamine, dopamine, and noradrenaline. In desensitization to bee stings, hymenoptera venom stimulates an allergenic response, decreases leukocyte sensitivity to the allergen, and increases the number of T-suppressor cells [14].

Bee venom (BV) (also called as apitoxin) has been widely used in the treatment of some immune-related diseases, as well as in recent times, in the treatment of tumours. Several cancer cells, including renal, lung, liver, prostate, bladder, and mammary cancer cells as well as leukaemia cells, can be the targets of bee venom peptides such as melittin and PLA2. Cell cytotoxic effects through the activation of PLA2 by melittin have been suggested to be the critical mechanism for the anti-cancer activity of BV [15].

Bee venom is a complex mixture of proteins, peptides and low molecular components. BV has numerous polypeptides, the main one being melittin, which shows anticancer effect.

Potential targets of BV

Cyclooxygenase 2 (COX-2)

The main target of non-steroidal anti-inflammatory drugs (NSAID) action is COX. COX-2 has been implicated in mammary carcinogenesis in several ways. The COX-2 expression has been localized to the epithelial compartment with no expression within the stromal component. Studies of breast cancer cell lines have provided data supporting the over expression of COX-2 in breast cancer. A study of the differential expression of COX-1 and COX-2 in human breast cancer cell lines found that in the MCF7 estrogen receptor-positive cell line, COX-2 was barely detectable, whereas in the highly invasive, metastatic cell line MDA-MB- 231 there was a low level of COX-1 but a high level of COX-2 expression [16].

It has been shown that melittin and bee venom prevent lipopolysaccharide and sodium nitroprusside-induced nitric oxide and prostaglandin E2 production via JNK pathway dependent inactivation of NF-κB, and suggests that inactivation of JNK pathways may also contribute to the anti-cancer effects of melittin and bee venom [17]**.**

Also, the anti-inflammatory activity of the n-hexane, ethyl acetate, and aqueous partitions from BV (*Apis mellifera*) has been studied using COX activity and pro-inflammatory cytokines (TNF-alpha and IL-1beta) production, *in vitro*. The aqueous partition of BV showed strong dose-dependent inhibitory effects on COX-2 activity (IC50 = 13.1 μg/mL). The aqueous partition was subfractionated into three parts by molecular weight differences, namely, B-F1 (above 20 KDa), B-F2 (between 10 KDa and 20 KDa) and B-F3 (below 10 KDa). B-F2 and B-F3 strongly inhibited COX-2 activity and COX-2 mRNA expression in a dose-dependent manner, without revealing cytotoxic effects. TNF-alpha and IL-1beta, are potent pro-inflammatory cytokines and are early indicators of the inflammatory process. The effects of three subfractions on TNF-alpha and IL-1beta production were investigated using ELISA method. All three subfractions, B-F1, B-F2 and B-F3, inhibited TNF-alpha and IL-1beta production. These results suggest that the pharmacological activities of bee venom on anti-inflammatory process include the inhibition of COX-2 expression and the blocking of pro-inflammatory cytokines (TNF-alpha, and IL-1beta) production [18].

Aromatase

Breast and endometrial cancers are highly responsive to oestrogen for growth evident by high concentrations of oestrogen receptors in these tissues. Malignant breast and endometrial tumours also produce large amounts of oestrogen locally via over expressing aromatase compared to their normal counterparts. In particular, aromatase over expression in breast cancer tissue has shown to be critical, since the use of aromatase inhibitors is clearly therapeutic in breast cancer [19].

Aromatase inhibitors (AIs) and inactivators interfere with the body's ability to produce oestrogen from androgens by suppressing aromatase enzyme activity. Three generations of AIs have been developed. Each successive generation has been associated with higher specificity for the aromatase enzyme, fewer adverse events, and greater suppression of aromatase activity [20].

Letrozole is a highly potent and selective AI that inhibits the enzyme activity of intracellular aromatase at the major sites where it is found, resulting in almost complete suppression of whole body aromatization. By effectively blocking oestrogen synthesis, letrozole inhibits the growth or induces the regression of hormone-responsive breast tumours *in vivo*. Letrozole induces complete regression of oestrogen-dependent, 9, 10-dimethylbenz-a-anthracene (DMBA)-induced mammary tumours in adult female rats. The chemical structure of letrozole (4, 40-[(1H-1, 2, 4-triazol-1-yl) methylene] bis-benzonitrile) is compared with other AIs. The nitrogen-containing structures like the imidazoles and the triazoles bind to the iron in the heme moiety of CYP-450, whereas the cyanobenzyl moiety present in the nonsteroidal AIs, such as letrozole, partially mimics the steroid backbone of the enzyme's natural substrate, androstenedione [21].

Melittin, present in BV which has a powerful haemolytic activity, [22] is a known activator of phospholipase A_2, suppresses the aromatase activity of physically dissociated trophoblast cells in a dose-dependent manner and acts as an aromatase inhibitor. Interestingly, melittin suppressed aromatase activity during short term (3 h) incubation but was ineffective on trophoblast cells from 24 h and 48 h incubations [23].

Free Radicals

Free radicals are ubiquitous in our body and are generated by normal physiological processes, including aerobic metabolism and inflammatory responses, to eliminate invading pathogenic microorganism. Targets of free radicals in inflammation include DNA, proteins, RNA and lipids. As free radicals can inflict cellular damage, several defences have evolved, both to protect our cells from radicals and to repair DNA damage [17].

The radical theory in human physiology claims that the active free radicals are involved in almost all the cellular degradation process and leads to cell death. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules and so to prevent such changes. Oxidative stress is thought to contribute to the development of chronic and degenerative diseases such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases [24]. Propolis, pollen and honey have the highest antioxidant activities [25].

Bee venom is a potent antioxidant, antifungal, antibacterial, anti-inflammatory, and possesses radioprotectant actions. It has been found to exert powerful actions as an antibacterial agent, anti-inflammatory, antiarthritic, antirheumatic, in neurodegenerative disease, as a cardiotonic, an antioxidant, and as a diaphoretic and diuretic. It has also appears to have a strong immunological agent, stimulating the body's protective mechanisms against disease. The Eclectic Botanical physicians considered it to be a potent alterative. This property of melittin, being an antioxidant, can be used to overcome the free radical effect [26].

uPA Receptors

In order for primary tumours to metastasise, tumour cells must undergo a complex series of steps, including detachment from the primary tumour, degradation of basement membranes, cell migration through the extracellular matrix (ECM), intravasation into the circulation, attachment and extravasation at a distant organ site, and cell division and growth of a new tumour colony. For all these steps, the urokinase plasminogen activator system (uPAS), along with members of the matrix metalloproteases (MMPs), has long known to play a key role. The urokinase-type plasminogen activator (uPA), when bound to its cellular receptor, uPAR, efficiently converts plasminogen into the serine protease plasmin, which then facilitates the release of several other proteolytic enzymes including gelatinase, fibronectin, fibrin, laminin, and latent forms of

collagenase needed to degrade physical barriers to cell movement. Binding of uPA to its receptor also triggers other important biological effects, including chemotaxis, migration, invasion, adhesion and proliferation [27].

The serpin family of serine protease inhibitors has generated a lot of interest in the past few years. One member in particular, maspin, has been under intense scrutiny as of late due to its seemingly growing potential in the cancer field as a therapeutic agent for breast and prostate cancers. Originally identified in a subtractive hybridization screen of breast cancer cells, maspin has since been classified as a putative tumour suppressor. It has been reported that maspin plays a novel role in the regulation of the urokinase-type plasminogen activator (uPA) and receptor (uPAR) protein system as it relates to hypoxia thereby illustrating yet another potential therapeutic pathway involving Maspin [28].

The function of the RSL domain of maspin on cell-ECM adhesion and tumor invasion has been explored. It is reported that that 1) the maspin RSL domain, but not the C-terminal region, is required, 2) the RSL peptide is sufficient for induction of increased cell-ECM adhesion of corneal stromal cells and carcinoma cells and inhibition of carcinoma cell invasion, and 3) the RSL can compete for specific binding of maspin to carcinoma cells [29].

Melittin ($C_{131}H_{229}N_{39}O_{31}$) is the main toxin in *Apis mellifera* (honeybee venom), and is characterized as a cationic, water-soluble peptide (+6 charge). The active peptide is originally released in the hive from its precursor, promelittin, until it is formylated. This biosynthesized melittin is comprised of a twenty-six amino acid sequence, with a hydrophobic amino-terminal and a hydrophilic carboxy-terminal. Melittin gains its biological significance in its antimicrobial, lytic abilities [30].

Comparison of the amino acid sequence of maspin with that of bee venom shows maximum similarity with melittin. Melittin will show anti-cancer effect against breast cancer and cell apoptosis will take place. This can happen because the amino acid sequence of the reactive site loop of maspin, which is a serine protease inhibitor, partially matches with melittin. It can associate either directly with the uPA/uPAR complex or indirectly through an integrin or other molecule and function as a tumour suppressor.

Snake Venom

It has been demonstrated that the snake venom extracted from *Walterinnesia aegyptia* (WEV) either alone or combined with silica nanoparticles enhanced the proliferation of mice immune cells and simultaneously decreased the proliferation of human breast carcinoma cell line (MDA-MB-231) [31].

Results have also demonstrated that CTX III inhibition of MDA-MB-231 cells may occur through inactivation of both PI3K/Akt and p38 MAPK signalling pathways, exerting inhibitory effects on NF-κB transcriptional factor, thereby decreasing the activity of MMP-9 and then posing an anti-metastatic effect in the cells [32].

Disintegrins

Disintegrins are low molecular weight peptides isolated from viper venom. Rubistatin is a MVD disintegrin cloned from a Crotalus ruber venom gland. Recombinant rubistatin (r-Rub) was cloned into a pET32b plasmid and expressed in reductase-deficient Escherichia coli. Its effect was tested on three cancerous cell lines and was found have an anti-proliferative, anti-migratory effect on the same and helping in the apoptosis of the same [33].

A component of snake venom has demonstrated its ability to inhibit cancer cell migration in two different cancer models. The protein, called contortrostatin, seems to block cell migration in a novel way. A minor component of the venom that worked as a disintegrin, and this protein was named contortrostatin. The integrins are a family of transmembrane receptor proteins that bind to components of the extracellular matrix. One of their functions is to grip the extracellular matrix, providing traction and allowing cells to migrate from one place to another. Researchers in a number of laboratories are focusing on one integrin in particular, called αvβ3. This integrin is present on the surface of cancer cells and is thought to be critical in metastasis.

Contortrostatin appears to block cell migration both by binding to a cell-surface protein in the integrin family, preventing it from gripping the extracellular matrix, and by scrambling signals to the cytoskeleton [34].

A therapeutic potential of disintegrin present in cobra snake venom contains a number of components with different pharmacological and biological activities were investigated. A study was designed to evaluate the effect of crude snake venom on a level of RNA and DNA of normal and breast cancer tissues *in vitro*. It was observed that in comparison to normal tissue the amount of RNA in cancerous tissue was higher about 84 %. And when a cancerous tissue was treated with snake venom (25 µg/ml) the content was reduced by 25 %. Similarly when same procedure was repeated for DNA contents the results showed similar pattern and amount of DNA increased in cancerous tissues by 57 %. The DNA amount is reduced with snake venom (25 µg/ml) by 95 %. Antitumour activity of contortrostatin (CN) is based on the high affinity interaction with several integrin displayed on both cancer cells and newly growing vascular endothelial cells. I. V administration of CN and long chain neurotoxin (LCN) effectively inhibits angiogenesis when compared with control. The diverse mechanisms of action provide CN with a distinct advantage over many other antitumour agents that only block a single pathway [35].

Cancer cell inhibitors

Cancer cell inhibitors, named atroporin and kaotree, having molecular weights of 35 KDa and 6 KDa have been isolated from the venoms of *crotalus atrox* and *naja kaouthia,* respectively, by fractionation on high pressure liquid chromatography. The purified atroporin and kaotree showed killing effects on various types of human (breast, colon, liver, ovary, etc.) And animal cancer cells in concentrations as low as 0.5µg/ml, and having no effect on normal mouse kidney, liver, spleen, and erythrocytes up to 5.0µg/ml. Both atroporin and kaotree prevent the formation of ascitic tumors caused by myeloma cells in balb/c mice. In addition, both atroporin and kaotree showed regression of ascitic tumors formed by myeloma cells. Atroporin and kaotree complement each other, as in combination they showed elevated anti-cancer activity *in vitro* and *in vivo* systems. However, atroporin and kaotree are immunologically distinct proteins showing no cross reactivity. Atroporin and kaotree, individually or in combination, have the potential for cancer biotherapy [36].

Indian monocellate cobra (*Naja kaouthia*) and Russell's viper (*Vipera russelli*) are common snakes of the East Indian sub-peninsula. The anticarcinogenic activities of their crude venoms were studied on carcinoma, sarcoma and leukemia models. Sub-lethal doses of venoms showed cytotoxicity on Ehrlich ascites carcinoma (EAC) cells in vivo. The venoms increased lifespan of EAC mice and strengthened the impaired host antioxidant system. Sarcoma formation in mice (3-methylcholanthrene induced) after venom treatment was significantly less ($p<0.005$). Histopathological examination of tumors showed tissue necrosis. The venoms displayed potent cytotoxic and apoptogenic effect on human leukemic cells (U937/K562). The venoms reduced cell proliferation rate ($p < 0.005$) and produced morphological alterations indicative of apoptosis induction. Different degree and nature of anticarcinogenic property of cobra and viper venoms may be attributed to the difference in their constituents [37].

Potential targets of snake venom

Anoikis

Echis carinatus (saw-scaled viper) produces potent hemorrhagic venom that causes the development of apoptotic and necrotic tissues. In one study, polyethyleneimine (PEI) was used to enhance cellular adherence, and to determine whether the substrate attachment influenced the survival of cells treated with crude *e. Carinatus* venom. Human embryonic kidney (HEK) 293t cells were grown for 18hr in tissue culture plates with or without PEI, and were then stimulated with crude *E. carinatus* venom for 3 or 12hr.

HEK 293t cells grown without PEI displayed a robust oxidative response to corresponding substrate detachment, loss of plasma membrane integrity and decreased cell viability. Cells grown on PEI adsorbed substrates demonstrated prolonged substrate attachment resulting in significantly higher cell viabilities. These observations suggest that the cytotoxicity of crude *E. carinatus* venom is dependent upon cellular detachment. Metallproteases, PLA2, and disintegrins from *E. carinatus* venom promoted apoptosis as a part of their pathological mechanisms. For example, *in vitro* studies using adherent cell cultures have shown exposure to purified disintegrins or snake venom metalloproteases (SVMP) induce cellular detachment. Interestingly, removal of the stimulants enables cellular reattachment and cellular survival, suggesting that disintegrins and SVMP induces a substrate dependent form of cell death, termed anoikis. Anoikis is important for maintaining tissue homeostasis and plays an essential role in the prevention of dissemination of cells to inappropriate sites, most notably during the metastatic process [38].

Anti-inflammatory and anti-neoplasmic activities of PLA2

Naturally occurring anti-toxic factors that neutralize PLA2 from the blood of both venomous and non-venomous animals have been isolated and studied. Snake PLA2 inhibitors (PLIS) are large multimeric, serum proteins that form soluble complexes with PLA2 enzymes, thereby inhibiting their actions. PLA2 isolated from *Bothrops neweidii* venom and Indian cobra, *Naja naja* venom, was found to be cytotoxic towards B16 F10 melanoma and ehrlich ascites tumour cells, respectively, suggesting its employment as an anticancer drug [39].

Platelet aggregation inhibitors

Many snake venom toxins affect platelet function. They can be grouped into a few major families, such as enzymes like serine proteinases, zinc-dependent pi-piv metalloproteinases of the reprolysin family and group II PLA2 isoenzymes as well as proteins with no enzymatic activity, such as c-type lectins, crisp and disintegrins. Of these, disintegrins and c-type lectins are considered as useful modulators of platelet function [39].

Indian monocellate cobra (*Naja kaouthia*) and Russell's viper (*Vipera russelli*) are common snakes of the East Indian sub-peninsula. The anticarcinogenic activities of their crude venoms were studied on carcinoma, sarcoma and leukemia models. Sub-lethal doses of venoms showed cytotoxicity on ehrlich ascites carcinoma (EAC) cells *in vivo*. The venoms increased lifespan of EAC mice and strengthened the impaired host antioxidant system. Sarcoma formation in mice (3 methylcholanthrene induced) after venom treatment was significantly less ($p < 0.005$). Histopathological examination of tumors showed tissue necrosis. The venoms displayed potent cytotoxic and apoptogenic effect on human leukemic cells (U937/K562). The venoms reduced cell proliferation rate ($p < 0.005$) and produced morphological alterations indicative of apoptosis induction. Different degree and nature of anticarcinogenic property of cobra and viper venoms may be attributed to the difference in their constituents [39].

Cytotoxins

Cytotoxins from cobra venom are known to manifest cytotoxicity in various cell types. It is widely accepted that the plasma membrane is a target of cytotoxins, but the mechanism of their action remains obscure. Using the confocal spectral imaging technique, it was shown for the first time that cytotoxins from cobra venom penetrate readily into living cancer cells and accumulate markedly in lysosomes. Cytotoxins CT1 and CT2 from *Naja oxiana*, CT3 from *Naja kaouthia* and CT1 from *Naja haje* are demonstrated to possess this property with respect to human lung adenocarcinoma A549 and promyelocytic leukaemia HL60 cells. Immobilized plasma membrane binding accompanies the internalization of CT3 from *Naja kaouthia* in the HL60 cells, but it is very weak for other cytotoxins. Detectable membrane binding is not a property of any of the cytotoxins tested in A549 cells. The kinetics and concentration-dependence of cytotoxin accumulation in lysosomes correlate well with their cytotoxic effects. On the basis of the results obtained, it was proposed that lysosomes are a primary target of the lytic action of cytotoxins. Plasma membrane permeabilization seems to be a downstream event relative to lysosome rupture. Direct damage to the plasma membrane may be a complementary mechanism, but its relative contribution to the cytotoxic action depends on the cytotoxin structure and cell type [40].

Scorpion Venom

Venom of some species of scorpions induces apoptosis and arrests proliferation in cancer cells. This is an important property that can be harnessed and can lead to isolation of compounds of therapeutic importance in cancer research.

The venom toxins isolated from Indian black scorpion, *Heterometrus bengalensis* show cytotoxic activity to leukemic cells and induced cell cycle arrest at G1 phase. Similarly, charybdotoxins isolated from the venom of *Leiurus quinquestraius herbraeus* arrest the cancerous cells in early G1 late G1 and S phase of cell cycle. A short peptide BMK-CBP isolated from Chinese scorpion *Buthus martensii* Karsch binds to cancer cell lines MCF-1 and inhibits cell growth. Similarly, BmYYAI, APBMV and BmKITal peptides isolated from red scorpion show anticancer activity by enhancing the activation and proliferation of lymphocytes and induction of immune function of WBCs in mice. Morespecially, *Buthus martensii* Karsch venom contains cholorotoxins which induce cell death in malignant glioma cells. These are used as novel tool for detection of skin cervical lung cancer, breast, prostate and testicular cancers and tumors. Similar activity is also showed by serine proteinase and hyaluronidase present in scorpion venom. Cercopins isolated from insects show anticancer activity [41].

Two novel peptides named neopladine 1 and neopladine 2 were purified from Tityus discrepans scorpion venom and found to be active on human breast carcinoma SKBR3 cells. Mass spectrometry molecular masses of neopladine 1 and 2 were 29918 and 30388 Da, respectively. Their N-terminal sequences were determined by Edman degradation. The peptides induced apoptosis of SKBR3 cells but had a negligible effect on non-malignant MA104 monkey kidney cells. Neopladine 1 and 2 induced 6.3 and 4.1% of SKBR3 apoptosis, respectively, in 5 h of exposure; the effect was larger with more prolonged exposures. Immunohistochemistry showed that neopladines bind to SKBR3 cell surface inducing FAS-L and BCL-2 expression [42].

Another study was done to examine the cytotoxic and anti-cancer properties along with addressing the plausible pathway followed by scorpion venom to reduce cell viability in SH-SY5Y and MCF-7 cells. Following exposure of cells with scorpion venom, cytotoxicity was estimated using MTT and lactate dehydrogenase assays. Apoptotic effects were measured by assessment of mitochondrial membrane potential, reactive nitrogen species, DNA fragmentation, and caspase-3 activity whereas antiproliferative effect was assayed using BrdU incorporation. The results indicate that scorpion venom causes suppression of proliferation by arresting S-phase and induction of apoptosis through increased nitric oxide production, caspase-3 activity and depolarization of mitochondrial membrane. Induction of apoptosis and arrest of DNA synthesis are critical determinant factors for development of anti-cancer drugs. These properties may lead to isolation of effective molecule(s) with potential anticancer activity from scorpion venom of *Androctonus crassicauda* [43].

MTT reduction assay has been used to measure cytotoxicity and confirmed with lactate dehydrogenase release following venom exposure. Inhibition of DNA synthesis in proliferating breast cancer cells was confirmed using immunocytochemical detection of BrdU incorporation. Results demonstrate that venom of *Odontobuthus doriae* not only induces apoptosis but lead to the inhibition of DNA synthesis in human breast cancer cells (MCF-7). Cell viability decreased with parallel increment of LDH release in dose dependent manner after treatment with varying concentrations of venom. These events were related to the depolarization of mitochondria and associated Caspase-3 activation following venom treatment in a concentration dependent manner. Finally, fragmentation of nuclear DNA following venom treatment confirmed the apoptotic property of the

said venom. These results suggest that venom of *O. doriae* can be potential source for the isolation of effective anti-proliferative and apoptotic molecules [43].

Spider Venom

Scorpion venoms are a complex mixture of a large variety of molecules and they play an important role in the defence and capture of prey. They contain mucopolysaccarides, phospholipases, hyaluronidases, protease inhibitors, low molecular weight molecules such as serotonin and histamine, histamine releasing peptides, inorganic salts, mucus, and many basic small proteins called neurotoxic peptides. The latter have specific interaction with ion channels, making scorpion venom capable of binding specifically to certain types of cells, such as cancer cells; therefore, this type of venom holds molecules that are of interest to the pharmaceutical industry in terms of drug design and development [44].

Spider venoms are a rich source of bioactive compounds with therapeutic potential. The venom of the spider *Macrothele raveni* potently suppresses cell growth in the myelogenous leukaemia K562 cell line in a dose and time-dependent manner with an IC (50) of 5.1 µg/mL. The results indicate that the venom of the spider *M. raveni* potently and selectively suppresses the growth of K562 cells by inducing apoptosis via caspase 3 and caspase 8 mediated signalling pathways [45].

A study was done to examine the effects of antitumor activity of the venom from the spider *Macrothele raven* (Araneae, Hexathelidae) on the human breast carcinoma cell line, MCF-7. The spider venom affected cell viability in a dose- and time-dependent manner as observed by [(3)H]-methyl thymidine incorporation assay. Cytotoxicity changes in MCF-7 cells caused by the spider venom at concentrations of 10, 20, and 40 mug/mL were determined by lactate dehydrogenase release assay. Flow cytometry showed that the spider venom induced apoptosis and necrosis of MCF-7 cells at these concentrations. MCF-7 cells treated with spider venom were accumulated on the G(2)/M and G(0)/G(1) phases. In addition, Western blotting analysis indicated that one of the pharmacological mechanisms of spider venom was to activate the expression of p21. *In vivo* examination of the inhibition of tumor growth in nude mice by the spider venom (at concentrations of 1.6, 1.8, and 2.0 mug/g mice) revealed that tumor size significantly decreases compared to controls by 21 days of treatment and at all points of analysis thereafter for 7 weeks ($p < 0.01$). [16].

Toad Venom

Bombesin

A study has demonstrated that bombesin/GRP antagonists reduces the expression of mRNA and protein levels of the most significant proangiogenic factors in breast cancer. These angiogenic and growth-promoting substances closely interact with each other. The tumor-inhibitory effect of bombesin/GRP antagonists appears to involve complex mechanisms. In this regard, it has been reported that RC-3095 and RC-3940-II can inhibit the growth of MDA-MB-435 cancer by downregulating mainly ErbB-2/HER-2, as well as by decreasing the expression of *c-jun* and *c-fos*oncogenes. Therefore, the mechanism responsible for the action of these antagonists on angiogenesis could be mediated by a downregulation of ErbB-2/HER-2, which would affect the pathways described above, and finally facilitate a decrease in the expression of the growth factors and MMPs as well as the vascular content. These findings confirm the merit of continued investigations of bombesin/GRP antagonists for the development of another approach to the management of breast cancer [46].

Brevinin

Brevinin-2R is a novel non-hemolytic defensin that was isolated from the skin of the frog Rana ridibunda. It exhibits preferential cytotoxicity towards malignant cells, including Jurkat (T-cell leukemia), BJAB (B-cell lymphoma), HT29/219, SW742 (colon carcinomas), L929 (fibrosarcoma), MCF-7 (breast adenocarcinoma), A549 (lung carcinoma), as compared to primary cells including peripheral blood mononuclear cells (PBMC), T cells and human lung fibroblasts. Jurkat and MCF-7 cells overexpressing Bcl2, and L929 and MCF-7 overexpressing a dominant-negative mutant of a pro-apoptotic BNIP3 were largely resistant towards Brevinin-2R treatment. The decrease in mitochondrial membrane potential, or total cellular ATP levels, and increased reactive oxygen species (ROS) production, but not caspase activation or the release of apoptosis-inducing factor (AIF) or endonuclease G (Endo G), are early indicators of Brevinin-2R triggered death. Brevinin-2R interacts with both early and late endosomes. Lysosomal membrane permeabilization inhibitors and inhibitors of cathepsin-B and cathepsin-L prevented Brevinin-2R-induced cell death. Autophagosomes have been detected upon Brevinin-2R treatment. Results have shown that Brevinin-2R activates the lysosomal-mitochondrial death pathway, and involves autophagy-like cell death [47].

Bufalin

Bufalin is the major digoxin-like immunoreactive component of Chan Su, a traditional Chinese medicine obtained from the skin and parotid venom glands of the toad. Bufalin and other bufadienolides are cardioactive C-24 steroids that exhibit a variety of biological activities, such as cardiotonic, anesthetic, blood pressure stimulation, respiration and antineoplastic activities. In terms of its anti-tumor activities, bufalin has demonstrated to induce growth inhibition, cell cycle arrest and apoptosis of tumor cells. Bufalin can be safely used for long periods without severe side effects. At high dosages, however, cardioactive steroids cause cardiac arrhythmia, breathlessness, seizure and coma. The structural similarity between bufadienolides and digoxin accounts for the toxic effects. Bufalin greatly sensitized ER-alpha-positive MCF-7 and ER-alpha-negative MDA-MB-231 human breast cancer cells to TRAIL-induced apoptosis. Enhanced apoptotic effects by TRAIL/bufalin combo were associated with augmentation of caspases activation. Further mechanistic investigation demonstrated that bufalin was able to significantly decrease Mcl-1 and BclXL expression levels, and inhibit the transcription factor STAT3. The important consequence of downregulation Mcl-1 in the enhancement action by combining bufalin with TRAIL was confirmed by either knockdown or overexpression of Mcl-1 approach [48].

Bufadienolides

The term “Bufodienolides” derives from the toad genus Bufo that contains bufadienolide glycosides, the suffix -adien- that refers to the two double bonds in the lactone ring, and the ending -olide that denotes the lactone structure. Bufadienolides are C-24 steroids; its characteristic structural feature is a doubly unsaturated six membered lactone ring having a 2-pyrone group attached at the C-17β position of the perhydrophenanthrene nucleus. On account of this chromophoric ring, they possess a characteristic UV absorption. Bufadienolides are a new type of natural steroids with potent antitumor activities. They have been reported to exhibit significant inhibitory activities against human myeloid leukemia cells (K562, U937, ML1, HL60), human hepatoma cells (SMMC7221), and prostate cancer cells (LNCaP, DU105, PC3). The activities are mediated by induction of cell apoptosis and cell differentiation, and the regulations of a variety of genes and proteins are involved in the process [49].

The death receptor ligand TRAIL is considered a promising candidate for cancer therapy because of its preferential toxicity to malignant cells. However its efficacy has been challenged by a number of resistance mechanisms. Therefore, agents that can overcome the resistance to enhance therapeutic efficacy of TRAIL are needed. In one study it was found that bufalin, bufotalin and gamabufotalin, key members of bufadienolides isolated from a traditional Chinese medicine ChanSu, significantly potentiated human breast cancer cells with different status of ER-alpha to apoptosis induction of TRAIL, as evidenced by enhanced Annexin V/FITC positive cells (apoptotic cells), cytoplasmic histone-associated-DNA-fragments, membrane permeability transition (MPT), caspases activation and PARP cleavage. Further mechanistic investigation demonstrated that bufalin was able to significantly decrease Mcl-1 expression and modestly decrease Bcl-XL expression level. Down-regulations of these anti-apoptotic proteins were well

correlated with inhibition of transcription factor STAT3 activation. The important consequence of down-regulation Mcl-1 in the enhancement action by combining bufalin with TRAIL was confirmed by either knockdown or overexpression of Mcl-1 approach. These findings for the first time provided strong evidences that bufadienolide compounds have excellent potential to be developed as a novel class of sensitizers of TRAIL [50].

CONCLUSION

Venom contains some broad spectrum of ion channel toxins that dominate medical importance. As with most species, there is evidence for novel activities in the presence of a high molecular weight, pulmonary edema inducing toxin is particularly intriguing. Some toxins appear to block cell migration by binding to a cell-surface protein in the integrin family, preventing it from gripping the extracellular matrix, and by scrambling signals to the cytoskeleton. It will be a long road for the venom from laboratory to clinic. To have results on cell-line or model organisms is completely different from pre-clinical or clinical trials and has to be conform with the pharmacological properties, which evidently are major stumbling blocks going from an interesting cell-biological observations to a market offering. The other major hurdle can also be the ethics or wild life regarding the use of venom. Nevertheless, the question remains, can this be developed as an anti-breast cancer drug?

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Freddie Bray, Jian-Song Ren, Eric Masuyer, Jacques Ferlay. Global estimates of cancer prevalence for 27 sites in the adult population in 2008. Int J Cancer 2013;132:1133–45.
2. Huang W, Seo J, Willingham SB, Czyzewski AM, Gonzalgo ML, *et al*. Learning from host-defense peptides: cationic, amphipathic peptoids with potent anticancer activity. PLoS ONE 2014;9(2): e903-97.
3. Pan H, Soman NR, Schlesinger PH, Lanza GM, Wickline SA. Cytolytic peptide nanoparticles ('NanoBees') for cancer therapy. Wiley Interdiscip Rev: Nanomed Nanobiotechnol 2011;3:318–27.
4. Park MH, Jo MR, Won D, *et al*. Snake venom toxin from *Vipera lebetina turanica* induces apoptosis in colon cancer cells via upregulation of ROS-and JNK-mediated death receptor expression. BMC Cancer 2012;12(1):228.
5. Song JK, Jo MR, Park MH, *et al*. Cell growth inhibition and induction of apoptosis by snake venom toxin in ovarian cancer cell via inactivation of nuclear factor κB and signal transducer and activator of transcription 3. Arch Pharm Res 2012;35(5):867-76.
6. Zhang XJ, *et al*. Development, characterization and anti-tumor effect of a sequential sustained-release preparation containing ricin and cobra venom cytotoxin. Pharmazie 2012;67(7):618-21.
7. Ye B, *et al*. Anti-tumor activity of CrTX in human lung adenocarcinoma cell line A549. Acta Pharmacol Sin 2011;32(11):1397-401.
8. Di Lorenzo L, Palmieri Chiara, Cusano Antonio, Foti Calogero. Cancer pain managment with a venom of blue scorpion endemic in cuba, called rhopalurus junceus " Escozul". Open Cancer J 2012;5:1-2.
9. Adam N Mamelak, Steven Rosenfeld, Richard Bucholz, *et al*. Phase i single-dose study of intracavitary-administered iodine-131-tm-601 in adults with recurrent high-grade glioma. JCO 2006;24(22):3644-50.
10. Ning YN, Zhang WD, Wu LC. Study on the mechanism of polypeptide extract from scorpion venom to promote the restraint of cyclophosphamide on Lewis lung cancer. Zhongguo Zhong Xi Yi Jie He Za Zhi 2012;32(4):537-42.
11. Huang WW, *et al*. Bufalin induces G(0)/G(1) phase arrest through inhibiting the levels of cyclin D, cyclin E, CDK2 and CDK4, and triggers apoptosis via mitochondrial signaling pathway in T24 human bladder cancer cells. Mutat Res 2012;732(1-2):26-33.
12. Qi F, *et al*. Induction of apoptosis by cinobufacini preparation through mitochondria-and Fas-mediated caspase-dependent pathways in human hepatocellular carcinoma cells. Food Chem Toxicol 2012;50(2):295-302.
13. Badr G, Garraud O, Daghestani M, Al-Khalifa MS, Richard Y. Human breast carcinoma cells are induced to apoptosis by samsum ant venom through an IGF-1-dependant pathway, PI3K/AKT and ERK signalling. Cell Immunol 2012;273(1):10-6.
14. Ellin JM, Batz F, Hitchens K. Natural medicines comprehensive database. stockton, ca: Therapeutic Res Faculty 2005:103–4, 681–3, 1034–5, 1088–9.
15. Oršolić N. Bee venom in cancer therapy. Cancer Metastasis Rev 2012;31(1-2):173-94.
16. G Davies, L A Martin, N Sacks, M Dowsett. Cyclooxygenase-2 (COX-2), aromatase and breast cancer: a possible role for COX-2 inhibitors in breast cancer chemoprevention. Ann Oncol 2002;13:669–78.
17. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer 2003;3(4):276-85.
18. Nam KW, Je KH, Lee JH, Han HJ, Lee HJ, Kang SK, Mar W. Inhibition of COX-2 activity and proinflammatory cytokines (TNF-alpha and IL-1beta) production by water-soluble sub-fractionated parts from bee (*Apis mellifera*) venom. Arch Pharm Res 2003;26(5):383-8.
19. Serdar E Bulun. Aromatase excess in cancers of breast, endometrium and ovary, J Steroid Biochem Mol Biol 2007;106(1-5):81–96.
20. C J Fabian. The what, why and how of aromatase inhibitors: hormonal agents for treatment and prevention of breast cancer. Int J Clin Pract 2007;61(12):2051–63.
21. Ajay S, Bhatnagar. The discovery and mechanism of action of letrozole. Breast Cancer Res Treat 2007;105:7–17.
22. Goran Gajski, Vera Garaj-Vrhovac. Melittin: a lytic peptide with anticancer properties. Environ Toxicol Pharmacol 2013;36(2):697-705.
23. Lin Show Whei. Arachidonic acid as a potential intracellular regulator of aromatase activity in human term trophoblast, Unpublished master's thesis for master's degree University of British Columbia, Canada
24. Lien Ai Pham-Huy, Hua He, Chuong Pham-Huy, Free Radicals. Antioxidants in disease and health. Int J Biomed Sci 2008;4(2):89–96.
25. Solange Teresinha Carpes, Rosicler Begnini, Severino Matias de Alencar, Maria Lúcia Masson. Study of preparations of bee pollen extracts, antioxidant and antibacterial activity. Ciênc Agrotec Lavras 2007;31(6):1818-25.
26. Gomase VS, Phadnis AC, Ghatak AA. Immunoproteomics approach for prediction of antigenic epitope of Tertiapin from *Apis mellifera.* Int J Drug Disc 2009;1(1):14-7.
27. Paul Bevan Carola Mala. The role of uPA and uPA inhibitors in breast cancer. Breast Care 2008;3(2):1–2.
28. Ming Zhang, Jeremy S Schaefer. Hypoxia effects: implications for maspin regulation of the uPA/uPAR complex. Cancer Biol Ther 2005;4(9):1033-5.
29. Chatri Ngamkitidechakul, *et al*. Sufficiency of the reactive site loop of maspin for induction of cell-matrix adhesion and inhibition of cell invasion. J Biol Chem 2003;278(34):31796–806.
30. Raghuraman H, Amitabha Chattopadhyay. Melittin: a membrane-active peptide with diverse functions. Biosci Rep 2007;27(4-5):189-23.
31. Al-Sadoon MK, Abdel-Maksoud MA, Rabah DM, Badr G. Induction of apoptosis and growth arrest in human breast carcinoma cells by a snake (walterinnesia aegyptia) venom combined with silica nanoparticles: crosstalk between bcl2 and caspase 3. Cell Physiol Biochem 2012;30(3):653-65.
32. Lin KL, Chien CM, Hsieh CY, Tsai PC, Chang LS, Lin SR. Antimetastatic potential of cardiotoxin III involves inactivation of PI3K/Akt and p38 MAPK signaling pathways in human breast cancer MDA-MB-231 cells. Life Sci 2012;90(1-2):54-65.
33. Carey CM, Bueno R, Gutierrez DA, Petro C, Lucena SE, Sanchez EE, Soto JG. Recombinant rubistatin (r-Rub), an MVD disintegrin, inhibits cell migration and proliferation, and is a

strong apoptotic inducer of the human melanoma cell line SK-Mel-28. Toxicon 2012;59(2):241-8.

34. Robert Finn. Snake Venom Protein Paralyzes Cancer Cells. J Natl Cancer Inst 2001;93(4):261-2.
35. Din Muhammad Shaikh, Rukhsana Jokhio. *In vitro* crude cobra snake venom significantly decreases the production of rna & dna in breast cancerous tissue. Pak J Physiol 2006;2(1).
36. B V LIPPS. Novel snake venom proteins cytolytic to cancer cells *in vitro* and *in vivo* systems. J Venom Anim Toxins 1999;5(2).
37. Anindita Debnath, *et al*. Venom of Indian monocellate cobra and Russell's viper show anticancer activity in experimental models. J Ethnopharmacol 2007;111(3):681-4.
38. Rebecca D Pierce, *et al*. Characterization of crude *Echis carinatus* venom-induced cytotoxicity in HEK 293T cells. J Venom Res 2011;2:59-67.
39. D C I Koh, A Armugam, K Jeyaseelan. Snake venom components and their applications in biomedicine. Cell Mol Life Sci 2006;63:3030–41.
40. Alexei V. Feofanov, *et al*. Cancer cell injury by cytotoxins from cobra venom is mediated through lysosomal damage. Biochem J 2005;390:11–8.
41. Ravi Kant Upadhyay. Animal proteins and peptides: Anticancer and antimicrobial potential. J Pharm Res 2010;3(12):3100-8.
42. D'Suze G, Rosales A, Salazar V, Sevcik C. Apoptogenic peptides from Tityus discrepans scorpion venom acting against the SKBR3 breast cancer cell line, Toxicon 2010;56(8):1497-505.
43. Zargan J, *et al*. Scorpion venom (Odontobuthus doriae) induces apoptosis by depolarization of mitochondria and reduces S-phase population in human breast cancer cells (MCF-7). Toxicol *In Vitro* 2011;25(8):1748-56.
44. Tiago Elias Heinen, Ana Beatriz Gorini da Veiga. Arthropod venoms and cancer. Toxicon 2011;57(4):497–511.
45. Liu Z, Zhao Y, Li J, Xu S, Liu C, Zhu Y, Liang S. The venom of the spider Macrothele raveni induces apoptosis in the myelogenous leukemia K562 cell line. Leuk Res 2012;36(8):1063-6.
46. A M Bajo, A V Schally, K Groot, K Szepeshazi. Bombesin antagonists inhibit proangiogenic factors in human experimental breast cancers. Br J Cancer 2004;90(1):245–52.
47. Saeid Ghavami, *et al*. Brevinin-2R [1] semi-selectively kills cancer cells by a distinct mechanism, which involves the lysosomal-mitochondrial death pathway. J Cell Mol Med 2008;12(3):1005-22.
48. Noriyuki Takai, *et al*. Bufalin, a traditional oriental medicine, induces apoptosis in human cancer cells. Asian Pacific J Cancer Prev 13:399-402.
49. Anjoo Kamboj, Aarti Rathour, Mandeep Kaur. Bufadienolides and their medicinal utility: a review. Int J Pharm Pharm Sci 2013;5(4):20-7.
50. Lia Hafiyani, Satoru Yokoyama, Sherif Abdelhamed, Yoshihro Hayakawa, Ikuo Saiki. Bufadienolides overcome trail resistance in breast cancer cells via jak-stat pathway. Altern Integ Med 2014;3:2.