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Anticancer, antimicrobial, and analgesic activities of spider venoms

Hassan M. Akef

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Spider venoms are complex mixtures composed of a variety of compounds, including salts, small organic molecules, peptides, and proteins. But, the venom of a few species is dangerous to humans. High levels of chemical diversity make spider venoms attractive subjects for chemical prospecting. Many spider venom components show potential activity against a wide range of human diseases. However, the development of novel venom-derived therapeutics requires an understanding of their mechanisms of action. This review will highlight the structures, activities and the possible mechanisms of action of spider venoms and their components against cancer, microbial infections, and pain.

1. Introduction

Natural toxins are a source of valuable pharmacological components that may play an important role in development of therapeutics. For example, venoms from some animals such as snakes and scorpions show a potential therapeutic activity against many diseases.[1–4] Interestingly, there are currently six US FDA-approved drugs derived from venom peptides or proteins with many more venom peptides currently in clinical trials or in preclinical development.[5]

Spiders are the most successful venomous taxa in terms of diversity, with an estimated 100 000 extant species.[6] Spider venoms are a complex mixture of low molecular weight organic components, proteins, polypeptides, neurotoxins, nucleic acids, free amino acids, inorganic salts and monoamines.[7,8] The primary function of spider venoms is to paralyze and kill prey. The major components of most spider venoms are peptides, with some species containing over 1000 unique peptides with masses ranging from 2 to 8 kDa.[9] It has been conservatively estimated that >10 million bioactive peptides are likely to be present in the venoms of spiders.[10] Spider-venom peptides display a diverse array of pharmacological activities directed towards modulating the activity of ion channels and receptors in the nervous system of prey and predators.[9] Although only a small number of spider venom peptides have been pharmacologically characterized, the array of known biological activities is impressive.[11] Most of the characterized peptides have high affinity for particular molecular targets and they are often selective for specific receptor subtypes.[9] This selectivity, functional diversity and biological stability make spider-venom peptides attractive for drug development.[12] For example, the range of pharmacological activities exhibited by disulfide-rich spider peptides is extraordinary and includes modulators of transient receptor potential (TRP) channels, mechanosensitive channels, acid-sensing ion channels (ASICs), glutamate receptors, glutamate transporters, calcium-activated potassium (K_{Ca}) channels and voltage-gated calcium (Ca_V) channels, voltage-gated sodium (Na_V) channels and voltage-gated potassium (K_V) channels.[13] In addition to peptides, the comparatively understudied small molecules and proteins of spider venoms may provide a source of novel drug leads.

Biologists who are interested in spider venom proteins can get important information from ArachnoServer (http://www.arachnoserver.org), which is a manually curated database containing information on the sequence, three-dimensional structure, and biological activity of protein toxins derived from spider venoms. Key features of ArachnoServer include a molecular target ontology designed specifically for venom toxins, current and historic taxonomic information and a powerful advanced search interface.[14]

Many spider venom components show potential therapeutic activity against a wide range of human diseases such as cancer,[15,16] microbial infections,[17,18] malaria,[19] arrhythmia,[20] and erectile dysfunction.[21] They also show analgesic activity[22] and potent neuroprotective efficacy.[23,24] In addition, many spider venom components show potential bioinsecticidal activity.[25] Furthermore, spider silk may be an interesting tool in biomedicine.[26] In this review I highlighted the structures, targets, and mechanisms of action of spider-venom components against cancer, bacterial, viral, and fungal infections, and pain.

National Organization for Research and Control of Biologicals (NORCB), Giza, Egypt. E-mail: hassan_akef@hotmail.com; Fax: +20-2-37628892; Tel: +20-2-37480478

2. Spider venoms in biomedicine

2.1. Anticancer activity

2.1.1. Whole venom as an anticancer agent. Zhang *et al.*[27] studied the effect of the *Lycosa vittata* spider venom on the K562, U937 human leukemia cell lines, the PC3 prostate cancer cell line, the MDA-MB-231 breast cancer cell line, and the control cell line HEK-293. The results showed that the venom caused an evident reduction of the viability of the four cancer cell lines in a dose-dependent manner. The IC_{50} values were calculated to be 31.6 ± 2.5 μg mL^{-1} for PC-3, 303.5 ± 3.8 μg mL^{-1} for U937, 338.2 ± 4.2 μg mL^{-1} for K562 and 396.1 ± 2.9 μg mL^{-1} for MDAMB-231. Interestingly, the venom showed a selective cytotoxicity on the five cell lines. The rank of susceptibility of the five cell lines to the venom was PC-3 > U937 ≈ K562 ≈ MDA-MB- 231 > HEK293 T. The venom had a very low inhibitory effect on HEK293 T cells with 500 μg mL^{-1} venom resulting in only less than 30% loss of cell viability; it exhibited the most potent inhibition on PC3 cells and the IC_{50} value of PC-3 is about 10-fold lower than that of other cancer cell lines. These data indicated that it is possible to identify anticancer compounds, especially agents selectively targeting PC-3, from this venom.[27]

The *Macrothele raveni* venom inhibited the proliferation and DNA synthesis of human hepatocellular carcinoma (BEL-7402) cells. It also induced apoptosis and cell cycle arrest in the G_0/G_1 phase and down-regulated C-myc in BEL-7402 cells.[28] Moreover, marked morphological changes, inhibition of proliferation, *caspase-3* upregulation, G_0/G_1 cell cycle arrest, and increasing the ratio of apoptosis and necrosis were observed in human cervix carcinoma (HeLa) after treatment with the *Macrothele raveni* spider venom. Also, an *in vivo* study was conducted on nude mice with cervical tumors (the tumor induced by injecting HeLa cells (200 μL) under the skin of nude mice with a concentration of 4×10^9 cells per L). The results showed that the tumor size significantly decreased after 3 weeks of treatment with the venom (1.0, 2.0, and 4.0 μg g^{-1}, tail vein injection) compared with the control group.[29]

The *Macrothele raven* venom significantly inhibits leukemic K562 cell growth with an IC_{50} value of 5.1 μg mL^{-1}. The venom also has a low inhibitory effect on human lymphocytes with an IC_{50} value ≈ 36.4 μg mL^{-1}, indicating that the venom is relatively selective for leukemic cells. Venom treated K562 cells showed typical morphological indicators of apoptosis including condensation of nuclei and fragmentation of DNA. Venom treatment also induced *caspase 3* and *caspase 8* activation in K562 cells and promoted poly-ADP ribose polymerase (PARP) cleavage, while *caspase 2*, *6* and *9* were not affected.[30]

Gao *et al.*[31] studied the effects of the *Macrothele raven* venom on the human breast carcinoma cell line, MCF-7. They found that treatment with the venom induced apoptosis and necrosis, and activated the expression of p21 in MCF-7 cells. Also, the spider venom caused a significant dose dependent accumulation of MCF-7 cells in the G_2/M and G_0/G_1 phases and reduced accumulation in the S phase. *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 μg g^{-1} mice; daily tail vein injection) revealed that the tumor size significantly decreased compared to the control after 21 days of treatment.

Macrothele raveni has cytotoxic, apoptotic, and necrotic activities on human squamous esophageal carcinoma cells (TE13) *in vitro*. The anti-cancer effects were likely achieved *via* decreasing DNA synthesis, inducing cell cycle arrest in the G_0/G_1 phase, increasing ROS levels, upregulating the expression of P21, and decreasing the cellular mitochondrial membrane potential (MMP) in TE13 cells. *In vivo* testing revealed that the tumor size was significantly decreased after 21 days of treatment with the venom.[32]

2.1.2. Peptides targeting cancer cells. Brachyin is a neurotoxin identified and characterized from venoms of the spider, *Brachypelma albopilosum*. There are six cysteines in its sequence, which form three disulfided bridges (the serine residue at the C-terminus is amidated). Zhong *et al.*[33] studied the effect of brachyin against human T cell lines Molt-4 and C8166, the bladder cancer cell lines BIU-87 and T24, and lung cancer cell lines A549 and Calu-6. It was observed that brachyin has significant cell proliferation inhibition activity against C8166, Molt-4, A549, BIU-87, T24, and Calu-6 cancer cell lines with IC_{50} values 1.5, 3.0, 6, 12, 12, and 24 μg mL^{-1}, respectively. Comparatively, human tumor cells especially those of solid tumor cell lines (Calu-6, BIU-87 and T24) were less sensitive to brachyin. Generally, C8166 cells were more sensitive than other cell lines to this peptide. Phase contrast photomicrographs showed that brachyin did not induce necrosis of toxicity damage and direct lysis in tumor cell lines.[33]

The lycosin-I, 24 amino acid peptide was isolated from the venom of the spider *Lycosa singorensis*; in contrast to most spider peptide toxins adopting an inhibitor cystine knot (ICK) motif, lycosin-I exhibits a linear amphipathic α-helical conformation and has a strong ability to inhibit tumor cell growth *in vitro* and *in vivo*.[16] Moreover, it was found that lycosin-I is able to interact with cell membranes and enter cell plasma to activate the mitochondrial death pathway to sensitize cancer cells for apoptosis, and up-regulates p27 to inhibit cell proliferation.[16] In recent studies, a strong interaction between lycosin-I and lipid membranes was observed by total internal reflection fluorescence microscopy. Lipid membranes induce the formation of amphiphilic-helix conformation of lycosin-I and subsequently stable aggregates of peptide molecules on the bilayer, which are believed to be crucial for the cell penetrating ability of lycosin-I.[34] More interestingly, lycosin-I showed a very low effect on normal cells, such as erythrocytes and Hek293t cells, when its concentration was much higher than the dosage that could kill cancer cell lines. Noncancerous cells were much less sensitive to lycosin-I possibly because of reduced interaction between lycosin-I and their membrane.[16]

Tan *et al.*[35] found that the lycosin-I-conjugated spherical gold nanoparticles (LGNPs) not only exhibited efficient cellular internalization efficiency toward cancer cells but also displayed unprecedented selectivity over noncancerous cells. Although LGNPs were removed from the living circulatory system *via* reticuloendothelial system-dominant clearance modes without

noticeable adverse effects to animals, they actually displayed active tumor-targeting effects and efficient accumulation in tumors *in vivo*. Furthermore, the potential application of this platform for cancer therapy was explored by lycosin-I-conjugated gold nanorods (LGNRs). LGNRs efficiently and selectively enter cancer cells, and kill cancer cells by the photothermal conversion effect under NIR irradiation (808 nm) *in vitro* and *in vivo*. Overall, the established LGNPs and LGNRs have great potential in cancer-targeting delivery and photothermal therapy.

Sun *et al.*[15] investigated the effects of recombinant Trx-jingzhaotoxin (JZTX)-III (originated from *Chilobrachy jingzhao* spider) on mouse hepatocellular carcinoma cell line Hepa1–6. It was observed that treatment with recombinant *E. coli* Trx-JZTX-III significantly decreased the proliferation of Hepa1–6 cells and the expression of the proliferating cell nuclear antigen (PCNA) protein, and inhibited the colony formation and the migration of malignant cells. Also, recombinant *E. coli* Trx-JZTX-III induced G0/G1 cell cycle arrest in Hepa1–6 cells.[15]

Gomesin is a cationic antimicrobial peptide isolated from hemocytes of a common Brazilian tarantula spider named *Acanthoscurria gomesiana*. It has two disulfide bridges ($Cys^{2,15}$ and $Cys^{6,11}$), and a β-hairpin structure. The peptide is highly amphipathic, with a hydrophobic face formed by residues Leu^5, Tyr^7, Val^{12}, and Tyr^{14}, and three hydrophilic regions containing positively charged and polar amino acids located at the N-terminus (Arg^3 and Arg^4), at the C-terminus (Arg^{16} and Arg^{18}), and within the noncanonical β-turn (Lys^8, Gln^9, and Arg^{10}).[36,37] Rodrigues *et al.*[38] found that topical treatment of subcutaneous murine B16F10-Nex2 melanoma with gomesin incorporated into a cream base significantly delayed tumor growth and also had significantly increased survival times as compared with controls. Apparently, the antitumor activity of gomesin (Gm) was due to a direct effect on tumor cells as Gm has a cytotoxic effect on B16F10-Nex2 murine tumor cells *in vitro* with the IC_{50} value below 5 μM. The β-hairpin structure of gomesin with disulfide bridges seemed essential for optimal activity. Gomesin apparently did not require a specific surface receptor to reduce tumor cell viability, because the enantiomer D-gomesin (D-Gm), which was synthesized employing D-amino acids and containing both disulfide bonds, was as cytotoxic as the native peptide implying that chiral recognition is not required for antitumor activity. A membrane permeabilizing activity was suggested, as gomesin bound to the cell membrane and cytoplasmic lactate dehydrogenase (LDH) was detected extracellularly. Repeated topical applications of Gm did not affect the peripheral healthy skin of peptide-treated mice.[38] Silva *et al.*[39] previously have shown a minimal hemolytic activity of gomesin *in vitro*, and Rodrigues *et al.*[38] also observed the complete absence of cytotoxicity of the peptide in thioglycollate-elicited peritoneal murine macrophages *in vitro*. Gomesin did not stimulate the production of nitric oxide in these macrophages. Seemingly normal cells may be resistant to gomesin unlike tumor cells and cultured endothelial cells. The *in vivo* antitumor effect of gomesin might also involve a cytotoxic effect on endothelial cells because cultured human endothelial cells were killed *in vitro* in a similar concentration range. Moreover, Gm at low concentrations could facilitate the internalization of macromolecules (immunoglobulins), which increased the cytotoxic effect.[38] Also, gomesin showed a direct cytotoxic effect against human breast adenocarcinoma (SKBr3), colon adenocarcinoma (LS180), human melanoma (SKMel 19 and A2058), and HeLa cell lines, the most sensitive being human melanoma A2058, with an IC_{50} value of 1.36 μM.[38]

Soletti *et al.*[40] found that gomesin or its analogues [$Ser^{2,15}$]-Gm and [$Ser^{6,11}$]-Gm, which contain only one disulfide bridge, were cytotoxic to human neuroblastoma SH-SY5Y and rat pheochromocytoma PC12 cancer cells and able to induce LDH release; however, [$Ser^{2,6,11,\ 15}$]-Gm, which lacks both disulfide bridges, did not exert any cytotoxic effect on the tested cancer cells. Gm induces necrotic cell death in SH-SY5Y cells. Gomesin evoked a rapid and transient elevation of intracellular calcium levels in Fluo-4-AM loaded PC12 cells, which was inhibited by nimodipine, an L-type calcium channel blocker. Preincubation with nimodipine also inhibited cell death induced by gomesin in SH-SY5Y and PC12 cells. Gomesin-induced cell death was prevented by the pretreatment with mitogen-activated protein kinase/extracellular regulated kinase (MAPK/ERK), Ca^{2+}/phospholipid-dependent protein kinase (PKC) or phosphatidylinositol-3-kinase (PI3K) inhibitors, but not with the cAMP dependent protein kinase A (PKA) inhibitor. In addition, gomesin generated reactive oxygen species (ROS) in SH-SY5Y cells, which were blocked with nimodipine and MAPK/ERK, PKC or PI3K inhibitors. Taken together, these results suggest that gomesin could be a useful anticancer agent, which mechanism of cytotoxicity implicates calcium entry through L-type calcium channels, activation of MAPK/ERK, PKC and PI3K signaling as well as the generation of ROS. It is interesting to note that gomesin and its analogues at their cytotoxic concentrations (0.5–10 μM) against tumor SH-SY5Y and PC12 cells did not produce significant lytic activity (<20%) on eukaryotic cells (human erythrocytes).[39]

McLachlan *et al.*,[41] Kekre *et al.*[42] and Siedlakowski *et al.*[43] studied the effects of Pancratistatin (PST) against human neuroblastoma (SHSY-5Y), human lymphoma (Jurkat), and breast cancer (MCF-7) cell lines, respectively. PST, a natural molecule isolated from the lily spider *Pancratium littorale*, was shown to have apoptotic activities specifically upon these cancer cells and not upon normal cells. Also, in combination with the anti-estrogen Tamoxifen, PST has a synergic effect against breast cancer cells.[43] In cancer cell lines, PST induced DNA fragmentation, permeabilization of mitochondria and activation of caspases, leading cells to apoptosis and also increasing ROS production. Interestingly, this molecule does not affect normal cells when compared to other drugs employed in clinical treatments of cancer, such as Etoposide (Vp-16) and Paclitaxel (Taxol).

2.2. Antimicrobial activity

2.2.1. Activity against bacterial and/or fungal infections

2.2.1.1. Whole venom. The crude venom isolated from *Lasiodora* sp. spider, found in northeastern Brazil, exhibited a

bactericidal effect against *Aeromonas* sp., *Bacillus subtilis*, and *Micrococcus luteus* and a fungicidal effect against *Candida parapsilosis* and *Candida albicans*. In addition, the venom exerted a bacteriostatic effect against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* and a fungistatic effect against *Candida tropicalis* and *Candida krusei*. The minimum inhibitory (MIC), minimum bactericidal (MBC), and minimum fungicidal (MFC) concentrations ranged from 3.9 to 500 μg mL^{-1}. On the other hand, the venom showed weak hemolytic activity against *Mus musculus* erythrocytes (EC_{50}: 757 μg mL^{-1}).[44]

Benli and Yigit[45] evaluated the antibacterial effects of the venom of *Agelena labyrinthica* (Clerck, 1757) (Araneae: Agelenidae) against several bacterial strains, specifically, testing 1/100, 1/10 and 1/1 fractions of diluted venom against these bacteria. The 1/1 and 1/10 diluted venom was effective against *Bacillus subtilis* RSHE, *E. coli* ATCC 25922, *Shigella* RSHE, *E. coli* RSHE, *S. aureus* ATCC 29213, and *P. aeruginosa* ATCC 27853. Also, 1/100 diluted venom was effective against *B. subtilis*. The venom-treated bacterial cells showed loss of cytoplasm and presented cell wall depression and shrinkage.

2.2.1.2. Antibacterial and/or antifungal peptides. Juruin is a 38-residue peptide, from the *Avicularia juruensis* spider venom, with three disulphide bridges conformed in an inhibitory cystine knot (ICK) motif, and a valine amidated at the C-terminus.[17] Based on the amino acid sequence, it is highly identical to the insecticidal peptides from the theraposid spiders *Selenocosmia huwena*, *Chilobrachys jingzhao*, and *Haplopelma schmidti* from China, indicating that they belong to a group of conserved toxins which are likely to inhibit voltage-gated ion channels. Juruin showed high antimicrobial activity against the following human clinical yeast isolates: *Candida krusei*, *C. glabrata*, *C. albicans* sp., *C. parapsilosis*, *C. tropicalis*, and *C. guilliermondii* and against the filamentous fungi *Aspergilus niger*.[17] The most sensitive strains were *Candida* spp. with MIC values ranging from 2.5–5 μM. Additionally, the filamentous fungi tested *Aspergilus niger* was highly sensitive to Juruin (MIC = 10 μM). However, Juruin did not show any antibacterial activity on Gram-positive strains *M. luteus*, *S. epidermidis*, and *S. aureus*, or on the Gram-negative strains *E. coli* and *P. aeruginosa*, even at a concentration as high as 100 μM.[17] Interestingly, Juruin lacks haemolytic activity on human erythrocytes at the antimicrobial concentrations. Juruin is a cationic AMP, and Lys^{22} and Lys^{23} show maximum positive charge localization that might be important for receptor recognition.[17]

Yan and Adams[46] identified two amphipathic α-helix antimicrobial pore-forming peptides, named lycotoxins I and II from the venom of the wolf spider *Lycosa carolinensis* (Araneae: Lycosidae). Both peptides potently inhibit the growth of bacteria (*E. coli* and *B. thuringiensis israelensis*) and *Candida glabrata* yeast.

Lycocitin 1 and 2 peptides (both are C-terminally amidated and positively charged) from the *Lycosa singoriensis* spider venom inhibit the growth of Gram-positive (*S. aureus*, *B. subtilis*) and Gram-negative (*E. coli*, *P. aeruginosa*) bacteria as well as that of fungi (*Candida albicans*).[47]

Seven Latarcin peptides named Ltc 1, Ltc 2a, Ltc 3a, Ltc 3b, Ltc 4a, Ltc 4b, and Ltc 5 are short linear cationic amphipathic α-helical peptides isolated from the venom of the spider *Lachesana tarabaevi*. They show pronounced antimicrobial effects against Gram-positive (*A. globiformis* and *B. subtilis*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacterial strains *in vitro*. Latarcins were also tested for antifungal activity against yeast cells (*P. pastoris* and *S. cerevisiae*), which were found to be much more resistant than bacteria, but their growth was still inhibited by higher concentrations of peptides. Erythrocytes are highly resistant to Ltc 3a, Ltc 3b, Ltc 4a, and Ltc 4b. Ltc 2a exhibited comparatively strong hemolytic activity, whereas Ltc 1 and Ltc 5 were moderately hemolytic. Planar lipid bilayer studies indicated that the general mode of action was scaled membrane destabilization at the physiological membrane potential consistent with the "carpet-like" model.[48]

Lazarev *et al.*[49] tested six antimicrobial peptides from the venom of *Lachesana tarabaevi* (latarcins 1, 2a, 3a, 4b, 5, and cytoinsectotoxin CIT 1a) for their ability to suppress *Chlamydia trachomatis* infection. HEK293 cells were transfected with plasmid vectors harboring the genes of the selected peptides. Controlled expression of the transgenes led to a significant decrease of *C. trachomatis* viability inside the infected cells. Interestingly, these recombinant AMPs showed a minimal cytotoxic effect on host cells.[49] Lazarev *et al.*[50] found that the CIT 1a peptide exerts its potent antichlamydial action at an early stage of the pathogen life cycle.

Cupiennin 1a (Cu 1a), a cytolytic peptide isolated from the venom of the spider *Cupiennius salei*, adopts α-helical conformation in the presence of negatively charged membranes and is attracted to the membrane by electrostatic interactions. It exhibits broad membranolytic activity towards bacteria (*E. coli*, and *S. aureus*), trypanosomes, and *Plasmodium falciparum* (K1 strain resistant to chloroquine and pyrimethamine), as well as human blood and cancer cells.[51] In analysing the cytolytic activity of synthesised all-D- and all-L-cupiennin 1a towards pro- and eukaryotic cells, a stereospecific mode of membrane destruction could be excluded. In the case of bacteria, both enantiomers are attracted by negatively charged phospholipids and lipopolysaccharides on the highly negatively charged outer membrane surfaces, resulting in α-helical conformation of the peptide and subsequent membrane disruption in a non-stereospecific manner. It was observed that reducing the overall negative charges of erythrocytes by partially removing their sialic acids or by protecting them with tri- or pentalysine results in reduced haemolytic activity of the peptide.[51] ^{31}P solid-state nuclear magnetic resonance (NMR) spectroscopy of different phospholipid bilayers with Cu1a points to a possible toroidal pore formation as the mechanistic basis of its antimicrobial effect.[52] Also, Kuhn-Nentwig *et al.*[53] reported that Cu 1a and Cupiennin 2a (Cu 2a) exhibit a comparable antimicrobial activity toward (*P. denitrificans* and *B. subtilis*) bacteria.

LyeTx I, an AMP isolated from the venom of *Lycosa erythrognatha*, has shown to be active against bacteria (*E. coli* and *S. aureus*) and fungi (*C. krusei* and *Cryptococcus neoformans*). It was observed that its antifungal activity is thus lower than its antibacterial activity when these organisms are taken into account. Also, LyeTx I is able to alter the permeabilisation of L-α-phosphatidylcholine-liposomes (POPC) in a dose dependent manner. It was observed that liposomes containing L-α-phosphatidylcholine : cholesterol (7 : 3 molar ratio) were approximately five times more resistant to the action of LyeTx I, when compared to those constituted only by L-α-phosphatidylcholine or of L-α-phosphatidylcholine : ergosterol (7 : 3 molar ratio). Interestingly, a weak haemolytic activity was observed in higher concentrations of the peptide. The secondary structure of LyeTx I shows a small random-coil region at the N-terminus followed by an α-helix that reached the amidated C-terminus, which might favour the peptide–membrane interaction.[54]

Gomesin is strongly effective against several Gram-positive and Gram-negative bacterial strains. Also, it has a marked activity against yeast strains such as *Candida albicans*, *C. tropicalis*, *Cryptococcus neoformans*, and *Saccharomyces cerevisiae* (MIC = 6.25 μM) and moderate activity against *C. glabrata* (MIC = 12.5–25 μM). Moreover, filamentous fungi such as *Alternaria brassicola*, *Aspergillus fumigatus*, *Fusarium culmorum*, *Fusarium oxysporum*, *Neurospora crassa*, *Nectria haematococca*, *Tricoderma viridae*, and *Tricophyton mentagrophytes* were highly sensitive to gomesin (MIC < 3.15 μM).[39] Furthermore, in association with fluconazole, gomesin concentrations with low antimicrobial activity (0.1–1 μM) inhibited *Cryptococcus neoformans* fungal growth and enhanced the antimicrobial activity of brain phagocytes. The mechanisms by which gomesin and fluconazole could act synergistically are still unknown.[55] It can be speculated that since gomesin can induce cell permeabilization in *C. neoformans*, the amount of fluconazole necessary to effectively reach the fungal cytoplasm and inhibit ergosterol synthesis could be decreased in the presence of the peptide.[55] Rossi *et al.*[56] reported that treatment with gomesin effectively reduced *Candida albicans* in the kidneys, spleen, liver and vagina of infected mice. Enhanced production of TNF-α, IFN-γ and IL-6 was detected in infected mice treated with gomesin, suggesting an immunomodulatory activity. After verifying that the gomesin treatment was effective against disseminated candidiasis in healthy mice, Rossi *et al.*[56] evaluated the activity of gomesin in immunosuppressed animals, as candidiasis is typically observed in immunocompromised hosts. They found that treatment with gomesin (5 mg kg^{-1}) showed no significant increase in survival compared to control animals. This suggests that the direct action of gomesin was not sufficient to control the infection and that immunomodulatory action is required to suppress the candidiasis. Treatment with fluconazole (20 mg kg^{-1}) also did not result in a significant increase in the survival of treated animals as compared to control animals. However, the combined treatment of 5 mg kg^{-1} gomesin and 20 mg kg^{-1} of fluconazole resulted in 23% survival of mice 30 days after infection.[56] This could be due to gomesin facilitating the entry of fluconazole into the yeast, thus leading to the survival of animals. Another hypothesis is that treatment with fluconazole, being fungistatic, would allow time for gomesin to act.[56] Interestingly, systemic administration of gomesin was also not toxic to the mice and no change either in haemoglobin levels or in the total number of leukocytes was observed in animals treated with gomesin. Overall, gomesin can be used as an alternative therapy for candidiasis, either alone or in combination with fluconazole. Gomesin's mechanism is not fully understood, but the peptide may act through the permeabilisation of the yeast membrane leading to death and/or releasing the yeast antigens that trigger the host immune response against infection.[56]

Lycosin-I showed rapid, selective and broad-spectrum bactericidal activity.[57] Also, both L- and D-lycosin-I displayed high antibacterial activities and rapid bactericidal effects against multidrug-resistant *Acinetobacter baumannii* (MDRAB) *in vitro*.[58] Moreover, the compounds retained their activity even at high salt (5 mM Mg^{2+} or Ca^{2+}) concentrations.[58]

The lycosin-II peptide, from the venom of the spider *Lycosa singoriensis*, contains 21 amino acid residue lacking cysteine residues and forms a typical linear amphipathic and cationic α-helical conformation. Lycosin-II displays a potent bacteriostatic effect on drug-resistant bacterial strains isolated from hospital patients, including multidrug-resistant *A. baumannii*.[59] The inhibitory ability of lycosin-II might derive from its binding to the cell membrane, because Mg^{2+} could compete with the binding sites to reduce the bacteriostatic potency of lycosin-II. Furthermore, lycosin-II has low hemolytic activity as compared with microbe cells; human erythrocytes are less sensitive to lycosin-II.[59]

Latroeggtoxin-IV, purified from the aqueous extract of the black widow spider eggs with a molecular weight of 3.6 kDa, was shown to be a broad-spectrum antibacterial peptide, showing inhibitory activity against Gram-positive bacterial strains (*S. aureus* and *B. subtilis*) and three Gram-negative bacterial strains (*E. coli*, *S. typhimurium* and *P. aeruginosa*), with the highest activity against *Staphylococcus aureus*.[60] Moreover, latroeggtoxin-IV is not a toxin for mice and cockroaches.[60] In order to further characterize Latroeggtoxin-IV, the peptide was subjected to sequencing by Edman degradation. Surprisingly, after the first Edman degradation cycle, it gave an obvious signal corresponding to Trp, and there were no other PTH-amino acid signals appearing in the consecutive cycles, suggesting the presence of a special structure, such as an intramolecular cycle, that prevented the sequencing from proceeding further.[60]

Oh-defensin, a defensin-like antimicrobial peptide from the venoms of the spider *Ornithoctonus hainana*, is composed of 52 amino acid residues including six Cys residues that possibly form three disulfide bridges. Oh-defensin exerted antimicrobial activities against Gram-positive bacteria, Gram-negative bacteria, and fungi. It showed potent antimicrobial activities against *S. aureus*, *E. coli*, *Bacillus dysenteriae*, and *C. albcans* with a MIC of 1.25 μg mL^{-1}. In addition, Oh defen-

sin has little hemolytic activity against rabbit red blood cells, only inducing 7% hemolysis at the concentration up to 200 μg mL^{-1}.[61]

2.2.1.3. Antibacterial and/or antifungal acylpolyamines. Pereira *et al.*[62] have isolated a 417 Da antibacterial molecule, named mygalin, from the hemocytes of the spider *Acanthoscurria gomesiana*. Mygalin was identified as bis-acylpolyamine *N*1,*N*8-bis(2,5-dihydroxybenzoyl)spermidine, in which the primary amino groups of the spermidine are acylated with the carboxyl group of the 2,5-dihydroxybenzoic acid. Mygalin was active against *E. coli* at 85 μM, this activity being inhibited completely by catalase. Therefore, the antibacterial activity of mygalin was attributed to its production of hydrogen peroxide (H_2O_2).[62]

The acylpolyamine called VdTX-I was isolated from the venom of the tarantula *Vitalius dubius*, and first described with activity as an antagonist of nicotinic cholinergic receptors.[63] Sutti *et al.*[64] reported that VdTX-I has antimicrobial activities against several bacterial and fungal strains. Also, the VdTX-I toxin has low hemolytic activity.

2.2.2. Activity against viruses. Dengue viruses are members of the *Flaviviridae* family. They are small, enveloped positive-sense RNA viruses transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes.[65] Dengue virus type 2 (DEN2), the most prevalent of the four serotypes, contains a single-stranded RNA and encodes a large single polyprotein precursor of 3391 amino acid residues which consists of three structural proteins (C, prM, and E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).[66] Processing of the polyprotein precursor to release mature viral proteins is mediated co- and posttranslationally by host proteases (furin and secretase) and the virus-encoded two-component protease NS2B-NS3pro.[67] The Ltc 1 peptide showed a significantly inhibitory effect against the dengue protease NS2B-NS3pro at 37 °C, a physiological human temperature, (IC_{50}, 12.68 ± 3.2 μM), and a greater inhibitory effect was observed at 40 °C, a temperature similar to a high fever (IC_{50}, 6.58 ± 4.1 μM). A greater reduction in viral load (p.f.u. mL^{-1}) was observed at simultaneous (0.7 ± 0.3 *vs.* 7.2 ± 0.5 control) and post-infection treatments (1.8 ± 0.7 *vs.* 6.8 ± 0.6 control) compared to the pre-infection treatment (4.5 ± 0.6 *vs.* 6.9 ± 0.5 control).[18] Treatment with the Ltc 1 peptide reduced the viral RNA in a dose-dependent manner with EC_{50} values of 8.3 ± 1.2, 7.6 ± 2.7 and 6.8 ± 2.5 μM at 24, 48 and 72 h, respectively.[18] The effects of the peptide on HepG2 cell proliferation were insignificant at 25 μM for 110 h because the cell index was similar to the untreated control cells (*i.e.* concentration of 25 μM was identified as the maximal non-toxic dose). Ltc 1 peptide may interrupt the dengue life cycle in HepG2 cells during post-translational processing of the polyprotein by inhibiting the dengue serine protease.[18]

Rothan *et al.*[68] reported the use of a new cost-effective approach involving production of a recombinant antiviral peptide-fusion protein that is scalable for the treatment of CHIKV infection. A peptide-fusion recombinant protein LATA-PAP1-THAN that was generated by joining the Latarcin (LATA, from the venom of the spider *Lachesana tarabaevi*) peptide with the N-terminus of the PAP1 (a ribosome-inactivating protein (RIP) from *Phytolacca Americana*) antiviral protein, and the Thanatin (THAN, a loop-structure peptide, isolated from the insect *Podisus maculiventris*) peptide to the C-terminus, was produced in *E. coli* as inclusion bodies. The antiviral LATA-PAP1-THAN protein showed 89.0% reduction of viral plaque formation compared with PAP1 (46.0%), LATA (67.0%) or THAN (79.3%) peptides alone.[68] The LATA-PAP1-THAN protein reduced the viral RNA load that was 0.89-fold compared with the untreated control cells. Also, PAP1 resulted in 0.44-fold reduction, and THAN and LATA resulted in 0.78-fold and 0.73-fold reductions, respectively. The LATA-PAP1-THAN protein inhibited CHIKV replication in the Vero cells at an EC_{50} value of 11.2 μg mL^{-1}, which is approximately half of the EC_{50} value of PAP1 (23.7 μg mL^{-1}) and protected the CHIKV-infected mice at the dose of 0.75 mg kg^{-1} (non-toxic dose). Overall, production of antiviral peptide-fusion protein in *E. coli* as inclusion bodies could accentuate antiviral activities, enhance cellular internalisation, and could reduce product toxicity to host cells and is scalable to epidemic response quantities.[68]

Acanthoscurrins are linear cationic peptides, from *Acanthoscurria gomesiana* spider venom, with a remarkable amino acid composition as only seven distinct amino acids composed these molecules: glycine (72–73%), leucine (12%), tyrosine (7%), lysine (4–5%), arginine (2%), valine (1%) and aspartic acid (1%). One particular feature of this sequence is the presence of three-fold repeats of 26 amino acids (GGGLGGGGLGGGGLGGGKGLGGGGLG). Two Gly-rich peptides, acanthoscurrins 1 and 2, have marked activity against *C. albicans* (MIC = 1.15–2.3 μM) and *E. coli* (MIC = 2.3–5.6 μM).[69]

2.3. Analgesic activity

Various ion-channels have been shown to play a significant role in the pathophysiology of pain including acid sensing ion channels, calcium (Ca_V) and sodium (Na_V) channels and purinergic receptors. Some spider venom components show potential analgesic activity *via* modulating these targets.

2.3.1. Na_V channel modulators. Voltage-gated sodium (Na_V) channels are transmembrane proteins that regulate the electrical properties of cells. There are nine mammalian subtypes denoted as $Na_V1.1$–$Na_V1.9$.[70] Several remarkable genetic studies led to the emergence of human $Na_V1.7$ ($hNa_V1.7$) as an analgesic target. Spider venoms are a rich natural source of $hNa_V1.7$ inhibitors that might be useful leads for the development of novel analgesics.

2.3.1.1. Peptides show analgesic activity. The β/ω-TRTX-Tp2a (ProTx-II) peptide from the *Thrixopelma prurient* spider venom, belonging to the inhibitory cystine knot (ICK) family of peptide toxins interacting with voltage-gated ion channels, has been reported as a blocker of Na_V 1.7 channels.[71] ProTx-II is at least 100-fold selective for human $Na_V1.7$ (with IC_{50} = 0.3 nM) compared with $Na_V1.2$, $Na_V1.3$, $Na_V1.4$, $Na_V1.5$, $Na_V1.6$, and $Na_V1.8$. However, ProTx-II has relatively high potency against

$Na_V1.2$ and $Na_V1.5$ resulting in high toxicity in rats (lethal to rats when injected intravenously at 1.0 mg kg^{-1} or by intrathecal administration of 0.1 mg kg^{-1}).[72] In contrast, beta-theraphotoxin-Gr1b (GsAFI), from the venom of the Chilean rose tarantula *Grammostola spatulata*, induced analgesia in a variety of rat pain models without any confounding side-effects. At present, the Na_V subtype selectivity of GsAFI is unknown.[73,74]

Phlotoxin 1 (PhlTx1), a 34-residue peptide toxin from the venom of a tarantula of the genus *Phlogiellus*, selectively inhibits $Na_V1.7$.[75] Moreover, a novel peptide, μ-theraphotoxin-Pn3a (Pn3a), isolated from the venom of the tarantula *Pamphobeteus nigricolor*, potently inhibits $hNa_V1.7$ (IC_{50} = 0.9 nM) with at least 40–1000-fold selectivity over all other Na_V subtypes.[76] Despite on-target activity in small-diameter dorsal root ganglia (DRG), spinal slices, and in a mouse model of pain induced by $Na_V1.7$ activation, Pn3a alone displayed no analgesic activity in formalin-, carrageenan- or (Freund's Complete Adjuvant) FCA-induced pain in rodents when administered systemically.[76] A broad lack of analgesic activity was also found for phlotoxin 1. However, when administered with subtherapeutic doses of opioids (oxycodone or buprenorphine) or the enkephalinase inhibitor thiorphan, these subtype-selective $Na_V1.7$ inhibitors produced profound analgesia.[76,77]

μ-TRTX-Tp1a (Tp1a), isolated from the venom of the Peruvian green-velvet tarantula *Thrixopelma pruriens*, is a Na_V 1.7 inhibitor.[78] Recombinant and synthetic forms of this peptide preferentially inhibited hNa_V 1.7 > hNa_V 1.6 > hNa_V 1.2 > hNa_V 1.1 > hNa_V 1.3 channels in fluorescence assays. Na_V 1.7 inhibition was diminished (IC_{50} = 11.5 nM) and the association rate decreased for the C-terminal acid form of Tp1a compared with the native amidated form (IC_{50} = 2.1 nM), suggesting that the peptide C terminus contributes to its interaction with hNa_V 1.7. Tp1a had no effect on human voltage-gated calcium channels or nicotinic acetylcholine receptors at 5 μM. Unlike most spider toxins that modulate Na_V channels, Tp1a inhibited hNa_V 1.7 without significantly altering the voltage dependence of activation or inactivation. Tp1a proved to be analgesic by reversing spontaneous pain induced in mice by intraplantar injection in OD1, a scorpion toxin that potentiates hNa_V 1.7. The peptide contains an ICK motif in which the Cys^2–Cys^{17} and Cys^9–Cys^{22} disulfide bonds form a 14-residue ring that is bisected by the Cys^{16}–Cys^{29} disulfide bond. In addition to the three disulfide bonds, the structure is stabilized by a large number of hydrogen bonds, including both the backbone and sidechain groups. The well-defined structure of Tp1a may help guide the development of improved $Na_V1.7$ inhibitors.[78]

GpTx-1, from the tarantula *Grammostola porteri* venom, shows potent activity on $Na_V1.7$ (IC_{50} = 10 nM) and promising selectivity against key Na_V subtypes (20× and 1000× over $Na_V1.4$ and $Na_V1.5$, respectively). It contains an inhibitory cystine knot (ICK) motif. Residues Trp^{29}, Lys^{31}, and Phe^{34} near the C-terminus are critical for potent $Na_V1.7$ antagonist activity.[79] GpTx-1 was found to significantly reduce OD1-induced spontaneous pain behaviors when administered locally, but lacked efficacy when delivered systemically. This is likely due to dose-limiting side effects, as the maximum tolerated systemic dose (0.1 mg kg^{-1}) may not have achieved sufficiently high concentration at peripheral nerve endings in the hind paw to inhibit $Na_V1.7$.[80]

2.3.1.2. Other candidate peptides. The spider venom peptide huwentoxin-IV (HwTx-IV) is a potent antagonist of hNav1.7. HwTx-IV is a 35 amino acid neurotoxic polypeptide secreted as a venom component of the Chinese bird-eating spider *Selenocosmia huwena* and is a member of the inhibitory cystine knot (ICK) super-family of mini-proteins.[81] Revell *et al.*[82] reported that the native residues Glu^1, Glu^4, Phe^6 and Tyr^{33} were revealed as important activity modulators and several peptides bearing mutations in these positions showed significantly increased potency on $hNa_V1.7$ while maintaining the original selectivity profile of the wild-type peptide on $hNa_V1.5$. Peptide **47** (Gly^1, Gly^4, Trp^{33}-HwTx) demonstrated the largest potency increase on hNav1.7 (IC_{50} = 0.4 ± 0.1 nM).[82] The HwTx-IV analogue (gHwTx-IV) showed improved affinity for the model lipid membranes. In addition, gHwTx-IV has increased activity at hNaV1.7 compared to HwTx-IV.[83] Agwa *et al.*[83] hypothesized that an increase in the affinity of HwTx-IV for lipid membranes is accompanied by improved inhibitory potency at hNaV1.7. These insights have the potential for translation to the design of analgesic inhibitors of $hNa_V1.7$.

The μ-TRTX-Hd1a (Hd1a) peptide, from the venom of the spider *Haplopelma doriae*, inhibited hNaV1.7 with a high level of selectivity over all other subtypes, except that hNaV1.1. Hd1a is composed of 35 amino acid residues with the sequence of ACLGFGKSCNPSNDQCCKSSSLACSTKHKWCKYEL. Hd1a is a gating modifier that inhibits hNaV1.7 by interacting with the S3b-S4 paddle motif in channel domain II. The structure of Hd1a, determined using heteronuclear NMR, contains an inhibitor cystine knot motif that is likely to confer high levels of chemical, thermal and biological stability.[84]

2.3.2. Acid sensing ion channel (ASIC) modulators. ASICs are proton-gated cation channels widely expressed throughout the central and peripheral nervous system. They belong to the ENaC (Epithelial Na^+ Channel)/DEG (Degenerin)/ASIC (Acid-sensing ion channel) superfamily of ion channels. Activation of ASICs is involved in pain perception, synaptic plasticity, learning and memory, fear, ischemic neuronal injury, seizure termination, neuronal degeneration, and mechanosensation. Therefore, ASICs emerge as potential therapeutic targets for manipulating pain and neurological diseases.[85] ASIC1a is one of the distinct ASIC subunits in CNS. The most selective and potent inhibitor of ASIC1a reported to date is psalmotoxin 1 (PcTx1), a disulfide-rich peptide isolated from the *Psalmopoeus cambridgei* spider venom.

PcTx1 inhibits homomeric ASIC1a and heteromeric ASIC1a/2b channels with IC_{50} values of 0.9 and 3 nM, respectively.[86] Intrathecal injection of PcTx1 reduced thermal, mechanical, chemical, inflammatory and neuropathic pain in rodents.[87,88] Interestingly, this peptide also increased endogenous Met-enkephalin levels in the cerebrospinal fluid.[87] Pc1a is only effective when administered intrathecally or by intracerebroventricular (ICV) injection, while intraperitoneal or sub-

cutaneous injections had no effect, as PcTx1 is a peptide.[87] PcTx1 is a 40-residue peptide stabilized by a classical inhibitor cystine knot motif, with a dynamic β-hairpin loop (βHL) protruding from the disulfide-rich core.[89,90] Site-directed mutagenesis combined with *in silico* docking revealed that PcTx1 binds to the acidic pocket of ASIC1a, with residues Trp^{24}, Arg^{26} and Arg^{27} within the βHL being critical for PcTx1 inhibition of the channel.[89] Mutation of Pro^{38} to Ala resulted in a fourfold decrease in potency, while substitution of Pro^{35} or Lys^{36} with → Ala leads to 2.7, 1.8 fold increase in ability to inhibit the rASIC1a channel, respectively.[91] Although crystal structures of the PcTx1 : cASIC1 complex revealed an extensive network of >50 intermolecular contacts, only ~50% of these interactions are essential for PcTx1 inhibition of rASIC1a.[91]

2.3.3. P2X Receptor modulators. P2XRs are ATP-gated non-selective cation channels. Seven mammalian purinergic receptor subunits, denoted P2X1 through P2X7, and several spliced forms of these subunits have been identified.[92] Pharmacological studies have shown that P2X3Rs is involved in acute pain, inflammatory pain, chronic neuropathic pain, visceral pain, migraine pain, and cancer pain.[93–95] Purotoxin-1 (PT1) is a potent and selective modulator of P2X3 isolated from the venom of the central Asian spider *Geolycosa sp*. PT1 is a 35-residue peptide including 8 cysteines involved in 4 intramolecular disulfide bridges. It adopts a rigid fold involving 2 short antiparallel β-strands (Lys^{20}–Lys^{22} and Val^{31}–Arg^{33}) and a number of β-turns crosslinked by 3 disulfide bridges (Cys^{3}–Cys^{16}, Cys^{10}–Cys^{21}, and Cys^{15}–Cys^{32}) that form the cystine knot structural motif characteristic of most known spider toxins.[96] It exerts an analgesic effect as it slows down the removal of desensitization of P2X3 receptors. Also, this peptide demonstrates potent antinociceptive properties in animal models of inflammatory pain.[96] An important feature of P2X3 receptors is that the high-affinity desensitization (HAD) which is a low nanomolar concentration of the agonist inhibits P2X3 receptor responses without evoking a macroscopic current.[97,98] Kabanova *et al.*[99] studied another polypeptide from the same spider venom named purotoxin-2 (PT2) and found that it also affected activity of mammalian P2X3 receptors. PT2 is composed of 64 amino acid residues with the sequence of AKACTPLLHDCSHDRHSCCRGDMFKYVCDCFYPE GEDKTEVCSCQQPKSHKIAEKIIDKAKTTL. Kabanova *et al.*[99] designed an experiment where the murine and human P2X3 receptors were heterologously expressed in cells of the CHO line, and nucleotide-gated currents were stimulated by CTP and ATP, respectively. They[99] found that both PT1 and PT2 negligibly affected P2X3-mediated currents elicited by brief pulses of the particular nucleotide. When subthreshold CTP or ATP was added to the bath to exert the high-affinity desensitization of P2X3 receptors, both spider toxins strongly enhanced the desensitizing action of the ambient nucleotides. At the concentration of 50 nM, PT1 and PT2 elicited a 3–4-fold decrease in the IC_{50} dose of ambient CTP or ATP. In contrast, 100 nM PT1 and PT2 negligibly affected nucleotide-gated currents mediated by murine P2X2 (mP2X2) receptors or mP2X2/mP2X3 heteromers.

2.3.4. Peptides modulating Ca_V channels and/or other pain targets. Voltage-gated calcium (Ca_V) channels are found in presynaptic nerve terminals where they allow the entry of calcium into cells through activation of Ca_V channels which directly affects membrane potential and contributes to electrical excitability, repetitive firing patterns, excitation-contraction coupling and gene expression. Native calcium channels are generally classified according to their electrophysiological and pharmacological properties as L-, N-, P-, Q-, or R-type channels (high-voltage-activated: ~−30 mV) or T-type channels (low-voltage-activated: ~−60 mV).[100–102] In fact, their presence in pain-modulation areas, such as the spinal cord, dorsal root ganglia, and brainstem, indicate the essential role of these types of VDCCs in the processing of pain related information in the central nervous system. Furthermore, VDCCs seem to be implicated in the central pain sensitization that occurs following nerve injury and during inflammatory states reviewed in ref. 103.

N-type calcium channels are often highly expressed both in dorsal root ganglia cell bodies and in the synaptic terminals projecting to the dorsal horn of the spinal cord.[104] These primary afferents are involved in the sensation of a number of noxious painful stimulations including mechanical, thermal and inflammatory.[22] N-type Ca_V channel inhibitor Ziconotide (Prialt), synthetic equivalent of omega-MVIIA (a component of the venom of the marine snail *Conus magus*), has been approved by the Food and Drug Administration (FDA) as an intrathecal analgesic in patients with severe chronic pain.[105] In addition, ω-conotoxin CVID, a specific blocker of N-type calcium channels isolated from the venom of the marine snail *conus catus*, as an analgesic drug is currently in Phase II clinical trials in Australia.[106]

Huwentoxin-XVI (HWTX-XVI), neurotoxin from the venom of the Chinese tarantula *Ornithoctonus huwena*, is specific for N-type calcium channels.[22] HWTX-XVI is composed of 39 amino acid residues including six cysteines that constitute three disulfide bridges. No toxic symptom was detected during a period of 48 h post-injection after direct toxin injection into mice (intra-peritoneal dose 1.127 μmol per kg body weight).

HWTX-XVI could almost completely block the twitch response of rat vas deferens to low-frequency electrical stimulation. Electrophysiological assay indicated that HWTX-XVI specifically inhibited N-type calcium channels in rat dorsal root ganglion cells (IC_{50} ~ 60 nM). The selectivity of HWTX-XVI for N-type over P/Q-type calcium channels is similar to that of HWTX-X or GVIA but higher than that of MVIIA. The toxin has no effect on voltage-gated T-type calcium channels, potassium channels or sodium channels. Intraperitoneal injection of the toxin HWTX-XVI to rats elicited significant analgesic responses to formalin-induced inflammation pain. Toxin treatment also changed withdrawal latency in hot plate tests. Intriguingly, intramuscular injection of the toxin reduced mechanical allodynia induced by incisional injury in the Von Frey test.[22] Overall, its analgesic effects, non-toxicity and complete reversibility make it an interesting tool

Table 1 List of some spider venom peptides that show potential therapeutic activities

Peptide	Origin	Amino acid sequence	AA	Reference or UniProKB ID
(A) Peptides show anticancer activities				
Brachyin[b]	*Brachypelma albopilosum*	CLGENVPCDKDRPNCCSRYECLEPTGYGWWYASYYCYKKRS	41	33
Lycosin-I[a]	*Lycosa singorensis*	RKGWFKAMKSIAKFIAKEKLKEHL	24	34
Gomesin[a]	*Acanthoscurria gomesiana*	ZCRRLCYKQRCVTYCRGR	18	39
(B) Peptides show antimicrobial activities				
Cupiennin 1a	*Cupiennius salei*	GFGALFKFLAKKVAKTVAKQAAKQGAKYVVNKQME	35	114
Juruin	*Avicularia juruensis*	FTCAISCDIKVNGKPCKGSGEKKCSGGWSCKFNVCVKV	38	17
Lycotoxin I	*Lycosa carolinensis*	IWLTALKFLGKHAAKHLAKQQLSKL-NH_2	25	46
Lycotoxin II	*Lycosa carolinensis*	KIKWFKTMKSIAKFIAKEQMKKHLGGE-OH	27	46
Lycocitin 1	*Lycosa singoriensis*	GKLQAFLAKMKEIAAQTL-NH_2	18	47
Lycocitin 2	*Lycosa singoriensis*	GRLQAFLAKMKEIAAQTL-NH_2	18	47
Ltc 1	*Lachesana tarabaevi*	SMWSGMWRRKLKKLRNALKKKLKGE-COOH	25	48
Ltc 2a	*Lachesana tarabaevi*	GLFGKLIKKFGRKAISYAVKKARGKH-COOH	26	48
Ltc 3a	*Lachesana tarabaevi*	SWKSMAKKLKEYMEKLKQRA-$CONH_2$	20	48
Ltc 3b	*Lachesana tarabaevi*	SWASMAKKLKEYMEKLKQRA-$CONH_2$	20	48
Ltc 4a	*Lachesana tarabaevi*	GLKDKFKSMGEKLKQYIQTWKAKF-$CONH_2$	24	48
Ltc 4b	*Lachesana tarabaevi*	SLKDKVKSMGEKLKQYIQTWKAKF-$CONH_2$	24	48
Ltc 5	*Lachesana tarabaevi*	GFFGKMKEYFKKFGASFKRRFANLKKRL-$CONH_2$	28	48
Cu 2a	*Cupiennius salei*	GFGTILKALAKIAGKVVKKLATKPGATYMLKENLK	35	53
CIT 1a	*Lachesana tarabaevi*	GFFGNTWKKIKGKADKIMLKKAVKIMVKKEGISKEEAQAKVDAMSKKQIRLYLLKYYGKKALQKASEKL-OH	69	49
LyeTx I	*Lycosa erythrognatha*	IWLTALKFLGKNLGKHLAKQQLAKL	25	54
Lycosin-II	*Lycosa singorensis*	VWLSALKFIGKHLAKHQLSKL	21	59
Oh-defensin	*Ornithoctonus hainana*	MLCKLSMFGAVLGVPACAIDCLPMGKTGGSCEGGVCGCRKLTFKILWDKKFG	52	61
Acanthoscurrin 1	*Acanthoscurria gomesiana*	DVYKGGGGGRYGGGRYGGGGGYGGGLGGGGLGGGGLGGGKGLGGGGLGGGGLGGGGLGGGGLGGGK GLGGGGLGGGGLGGGGLGGGGLGGGKGLGGGGLGGGGLGGGRGGGYGGGGGYGGGYGGGYGGGKYK	132	69
Acanthoscurrin 2	*Acanthoscurria gomesiana*	DVYKGGGGGRYGGGRYGGGGGYGGGLGGGGLGGGGLGGGKGLGGGGLGGGGLGGGGLGGGGLGGGKGL GGGGLGGGGLGGGGLGGGGLGGGKGLGGGGLGGGGLGGGRGGYGGGGGYGGGYGGGYGGGKYK	130	69
(C) Peptides with analgesic activity				
1. Peptide targeting hNa_V 1.7				
ProTx-II	*Thrixopelma prurient*	YCQKWMWTCDSERKCCEGMVCRLWCKKKLW	30	71
GsAFI	*Grammostola spatulata*	YCQKWLWTCDSERKCCEDMVCRLWCKKRL	29	P61408
TRTX-Pn3a	*Pamphobeteus nigricolor*	DCRYMFGDCEKDEDCCKHLGCKRKMKYCAWDFTFT	35	76
Phlotoxin 1	*Phlogiellus* genus	ACLGQWDSCDPKASKCCPNYACEWKYPWCRYKLF	34	76
Tp1a	*Thrixopelma pruriens*	DCLKFGWKCNPRNDKCCSGLKCGSNHNWCKLHL	33	78
GpTx-1	*Grammostola porteri*	DCLGFMRKCIPDNDKCCRPNLVCSRTHKWCKYVF-NH_2	34	79
2. ASICs modulators				
PcTx1	*Psalmopoeus cambridgei*	EDCIPKWKGCVNRHGDCCEGLECWKRRRSFEVCVPKTPKT	40	P60514
3. P2X Receptors modulators				
PT1	*Lycosa kazakhstanicus*	GYCAEKGIRCDDIHCCTGLKCKCNASGYNCVCRKK	35	96
4. Peptides modulating Ca_V channels and/or other pain targets				
HWTX-XVI	*Ornithoctonus huwena*	CIGEGVPCDENDPRCCSGLVCLKPTLHGIWYKSYYCYKK	39	22
Phα1β	*Phoneutria Nigriventer*	ACIPRGEICTDDCECCGCDNQCYCPPGSSLGIFKCSCAHANKYFCNRKKEKCKKA	55	115
Tx3-3	*Phoneutria nigriventer*	GKCADAWESCDNYPCCVVNGYSRTCMCSANRCNCDDTKTLREHFG	45	116

AA, number of amino acids. [a] Peptides show both anticancer and antimicrobial activities. [b] Peptides show both anticancer and analgesic activities.

for developing novel drugs for the treatment of N-type calcium channel-related diseases.

Tx3-3, a peptide toxin isolated from the *Phoneutria nigriventer* venom, inhibits high-voltage-dependent calcium channels (VDCC), preferentially P/Q and R-type VDCC.[107] Dalmolin *et al.*[103] tested the effects of Tx3-3 in animal models of nociceptive (tail-flick test), neuropathic (partial sciatic nerve ligation and streptozotocin-induced diabetic neuropathy), and inflammatory (intraplantar Freund's Complete Adjuvant) pain. In the tail-flick test, both intrathecal (i.t.) and intracerebroventricular (i.c.v.) injection of Tx3-3 in mice caused a short-lasting effect (ED_{50} and 95% confidence intervals of 8.8 [4.1–18.8] and 3.7 [1.6–8.4] pmol/site for i.t. and i.c.v. injection, respectively), without impairing motor functions, at least at doses 10–30 times higher than the effective dose. By comparison, ω-conotoxin MVIIC caused significant motor impairment at doses close to the efficacious dose in tail flick test. Tx3-3 showed a long-lasting antinociceptive effect in neuropathic pain models. Intrathecal injection of Tx3-3 (30 pmol per site) decreased both mechanical allodynia produced by sciatic nerve injury in mice and streptozotocin-induced allodynia in mice and rats. Conversely, Tx3-3 did not prevent or reverse FCA-induced mechanical allodynia.[103] Overall, this peptide toxin holds promise as a novel therapeutic agent for the control of neuropathic pain.

Phα1β is a peptide purified from the venom of the armed spider *Phoneutria nigriventer* that reversibly inhibits voltage-gated calcium channels (VGCCs), with some selectivity for N-type VGCCs.[108] This peptide exerts antinociceptive effects in several preclinical pain models, such as post-surgical, neuropathic and cancer-related pain.[109–111] Moreover, Phα1β was effective in potentiating the analgesia caused by a single dose of morphine as well as in reducing tolerance and the adverse effects induced by repeated administration of morphine, indicating its potential use as an adjuvant drug in combination with opioids.[112] Recently, Tonello *et al.*[113] reported that Phα1β and its recombinant form (CTK 01512–2) are selective and potent TRPA1 (Transient receptor potential ankyrin-1) channel antagonists with antihyperalgesic effects in a relevant model of neuropathic pain.

2.3.5. Other analgesic peptides. Some peptides show analgesic activity but their mechanism of action is not reported. For example, Brachyin showed significant analgesic effects in mice models including abdominal writhing induced by acetic acid and formalin-induced paw licking tests.[33]

3. Conclusion

The venoms of spiders contain a variety of chemical compounds. Many spider venom components show analgesic activity and a potential therapeutic activity against diseases such as cancer and bacterial, fungal, and viral infections (Table 1). Based on the mechanisms of action and structure–function relationship studies, the scientists can design novel therapeutic agents. Finally, the clinical application of spider venom-derived components for the treatment of these diseases yet needs to move forward.

Conflicts of interest

There are no conflicts of interest to declare.

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