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Research on Peptide Toxins with Antimicrobial Activities

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Abstract

Microbes may quickly acquire resistance to the antibiotics used, since antibiotic agents are now extensively applied in treatment of infectious diseases in clinic. Novel antimicrobial agents are therefore urgently needed to overcome the resistance issue. Up to date, great efforts have been made in identifying the new antimicrobial agents from natural resources as alternative approach. Peptide toxins existing in animal venoms are demonstrated in recent studies to be of particular importance in prey capture and defense. Due to the diverse structures and bioactivity of peptide toxins, certain peptide toxins have exhibited broad antimicrobial activity, which may have potential to be developed either as new antimicrobial agents or as template for drug design. In this paper, peptide toxins with antimicrobial potential, as well as their biochemical derivatives from various animals, for instance scorpions, snakes, spiders, ants and others were carefully reviewed and discussed.

Keywords: Animal venom; Peptide toxin; Antimicrobial activity

Introduction

Healthy status of human being caused by microbial infection varies from mild to severe, even fatal, in terms of severity. In our daily life, a variety of microbes are widespread, resulting in many diseases (Table 1). Antibiotics, as the most conventional therapy, play a vital role in the treatment of microbial infection. There is no doubt that antibiotics still remain as the most effective agents for infectious diseases. As antibiotics are applied extensively, however, genetic alterations give rise to changes in the expression of the cellular targets of antibiotics, affecting the signaling pathways. Response of microbe to stimuli and action of antibiotics are then altered. Eventually, microbes acquire resistance to antibiotics after a period of clinical use. Since microbial infections are common, developing alternative strategy to infection becomes urgent. The biodiversity of component of venoms offers a useful tool from which new antimicrobial agents may be developed.

Venoms are naturally producing and secreting by a great number of organisms for defense and/or capture preys [1]. In general, venom is comprised of many bioactive chemical substances, such as enzymes, proteins, peptides and other small chemical entities. Among the molecules, peptide toxins are of particular interests. Special considerations have been given by pharmaceutical scientists and industry, since certain molecules may have great potential to become new therapeutic agent. The known targets of the toxin isolated from venoms include ion channels, acetylcholine receptors, acetyl cholinesterase, plasma membranes, metalloproteases and others [1-3]. Generally, peptide toxins are synthesized in the venomous ducts of poisonous creatures, such as snakes, frogs, spiders, marine snails, ants, wasps, bees, and centipedes. Peptide toxins are small, ranging from 8-70 amino acids with diverse activity for treatment of pain, diabetes, epilepsy, cardiovascular disorders, cancer, neurological disorders, multiple sclerosis and microbial pathogen infections [1].

Peptide toxins with antimicrobial activities

Scorpion peptide toxins: Scorpions are venomous arthropods, which are distributed globally, in paritulcar in tropical and climatic region. Venoms of scorpions have been used in medicine for over thousands of years. Numerous biological molecules such as nucleotides, biogenic amines, enzymes, and peptides/proteins were isolated from the venom and identified. Currently, hundreds of peptide toxins have been characterized. The peptide toxins in scorpion venom exhibit their bioactivities through blocking ion channel or modifying its function. Their potential therapeutic applications include antimicrobial activity, anticancer activity, treating autoimmune diseases, cardiovascular effects and others [3].

Several peptide toxins have already shown potent antimicrobial activity and are under different stages of development. The earliest peptide toxin ever studied was androctonin, which was isolated

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from the venom of scorpion *androctonus australis*. Androtonin showed potent antibacterial activity against both gram-positive and gram-negative bacteria [4]. In addition, Moerman et al. [5] also reported potent antifungal activity of androtonin. Powers et al. [6] reported the antibacterial activity of other peptide toxins, for example parbutoporin (isolated from scorpion *Parabuthus schlechteri*) and opistoporins (isolated from scorpion *Opistophthalmus carinatus*). These peptide toxins targeting at G proteins and are different from the typical AMPs with membrane lytic activity. Imperatoxin-I, a long chain toxin composed of 75 amino acid residues and cross linked by three disulfide bridges could inhibit protozal infection to human [7].Recently, more peptide toxins with antimicrobial activity were characterized. TstH (Tityus stigmurus Hypotensin), was deduced from the transcriptome of T. stigmurus venom gland and exhibited antifungal activity [8]. Valdez-Velazquéz LL et al. [9] isolated two peptides from venom from the scorpion Centruroides tecomanus which showed potent antibacterial activity. VpAmp1.0 and VpAmp2.0 were two new non-disulfide bound peptides, isolated from scorpion Vaejovis punctatus. They could effectively inhibit the growth of both Gram-positive (S*taphylococcus aureus* and *Streptococcus agalactiaea*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria, yeasts (*Candida albicans* and *Candida glabrata*) and two clinically isolated strains of Mycobacterium tuberculosis-including a multi-drug resistant one [10]. Opisin was a peptide toxin from scorpion Opistophthalmus glabrifrons. It is a cationic, amphipathic, and α-helical molecule with 19 amino acid residues without disulfide bridges. Opisin is able to potently inhibit the growth of the tested Gram-positive bacteria. However, it possesses much lower activity against the tested Gram-negative bacteria and a fungus [11]. Stigmurin was isolated from Brazilian yellow scorpion *Tityus stigmurus* venom gland, which was an underexplored source for peptide toxin. It showed antibacterial and antifungal activity [12]. Ctriporin isolated from the venom of the scorpion *Chaerilus tricostatus*, shows a broad-spectrum of antimicrobial activity and is able to inhibit antibiotic resistant pathogens, including Methicillin resistant Staphylococcus aureus, Methicillin Resistant Coagulase-negative Staphylococcus, and Penicillin Resistant Staphylococcus epidermidis strains [13]. Hp1404 was identified from the scorpion *Heterometrus petersi*i, which is an amphipathic α-helical peptide and has a specific inhibitory activity against gram-positive bacteria including methicillin-resistant Staphylococcus aureus [14].

Snake peptide toxins: In general, snakebites are extremely harmful to human beings, leading to severe symptom or even death. Similar to scorpion venoms, snake venoms have also been used in treatment of various diseases. The component and functions of snake venom have now been uncovered by investigation. Snake venoms were comprised of a variety of peptides/proteins and small molecules, which exert neurotoxic, cytotoxic, cardiotoxic, myotoxic and enzymatic activities. The peptide toxins in snake venom which have potential therapeutic properties against microbe infections are discussed.

The compounds in snake venom which exhibit antimicrobial activity are mainly enzymes such as phospholipase A2, metalloproteinases and L-amino acid oxidases and peptides. However the peptide toxins with antimicrobial activity in snake venom remain unclear. The reported peptide toxins include two neurotoxic Cardiotoxins (cardiotoxin 3 and tonxin γ) and crotamine, whose mechanism of action was similar to AMPs killing bacteria by disrupting their membrane. Cardiotoxin 3 isolated from *N. naja atra* (Taiwan cobra) showed potent antibacterial activity against both Gram positive and Gram negative bacteria. However, the antibacterial activity against *S. aureus* was much more potent than that against *E. coli,* which may be attributed to different composition of the cell membrane of *E. coli* and *S. aureus* [15]. Tonxin γ isolated from Naja nigricolliss showed potential antibacterial activity against *E. coli* and *S. aureus*, since it showed membrane perforating activity on the *E. coli* and *S. aureus* mimicking membrane [16]. Crotamine, from the venom of South American rattlesnake, shares gene ancestry with the vertebrate β-defensins and exhibits broad spectrum of antimicrobial activity. It demonstrated potent antibacterial activity against *E. coli.* However, the activities against other gram-negative bacteria and Gram-positive were limited [17]. Antifungal activity against *Trichosporon spp*., *Cryptococcus neooofffformans*, and *Candida spp* was discovered. But weak against the filamentous fungus *Aspergillus fumigatus* and *Trichophyton rubrum* [18]. Crotamine also presented in vitro antileishmanial activity against *Leishamania amanuensis* in solution form and encapsulated in biodegradable microparticles [19].

Spider peptide toxins: Spiders are biologically and ecologically diverse on the planet with almost 40, 000 species, several are truly dangerous to human. Almost all of spiders possess a venom apparatus and secret toxins to paralyze and kill their prey. Spider venoms are complex cocktails of toxins, comprising small molecules and peptides/proteins, including inorganic ions and salts, free acids, glucose, amino acids, biogenic amines and neurotransmitters. Recently, the diverse peptide toxins in spider venom have attracted great attention as promising drug leads and excellent pharmaceutical and biological tools. Most spider peptide toxins contain multiple disulfide bonds and act as neurotoxins targeting at different ion channels, receptors and nervous systems. There is also a special type of spider toxins with deficiency of cysteine residues, which gained an increasing recognition. These toxins are amphipathic and positively charged, which exhibit strong antimicrobial activity.

Lycotoxin I and Lycotoxin II were the initial reported peptide toxins isolated from the venom of wolf spider *lycosa carolinensis*. The lycotoxins may play a dual role in spider-prey interaction, functioning both in the prey capture strategy as well as to protect the spider from potentially infectious organisms arising from prey ingestion [20]. Kozlov et al. [21] reported seven linear peptide toxins named latarcins, from the venom of the spider *Lachesana tarabaevi*, presenting antimicrobial activity against Gram-positive and Gram-negative bacteria, and yeast. Oxyopinins, from the crude venom of the wolf spider *Oxyopes kitabensis*, demonstrated high antimicrobial activity against a range of Gram-positive and Gram-negative bacteria [22]. Cupiennin 1a was a potent venom component of the spider *Cupiennius salei*. It exhibited multiple activities, including antimicrobial activity [23]. LyeTx I [24], from the venom of wolf spider *Lycosa erythrognatha*, showed antimicrobial activity against bacteria (*Escherichia coli* and *Staphylococcus aureus*) and fungi (*Candida krusei* and *Cryptococcus neoformans*). Ep-AMP1 from *Echinopsis pachanoi*, was the first cystine knot peptide from Cactaceae (cactus) family.Its activity was more than 500 times higher against bacterial than that against eukaryotic cells [25]. The latest reported spider peptide toxin was Lycosin-II, which was isolated the venom of spider *Lycosa singoriensis*. It contains 21 amino acid and shows potent bacteriaostatic effect on the clinically isolated drug resistant bacteria, including multidrug resistant *A. baumannii* [26].

Ant peptide toxins: Ants are one of the most abundant groups

Table 1: The microbial causes of some common diseases and infections.

Peptide toxin	Bacteria	Fungus	Protozoa	Virus
Athlete's foot		♦		
Chickenpox				♦
Common cold				♦
Diarrheal diseases	♦		♦	♦
Flu (Influenza)				♦
Genital herpes				♦
Malaria			♦	
Meningitis	♦			♦
Pneumonia	♦	♦		♦
Sinusitis	♦	♦		
Skin diseases	♦	♦	♦	♦
Tuberculosis	♦			
Urinary tract infection	♦			
Vagina infections	♦	♦	♦	
Viral hepatitis				♦

species of venomous organisms, with over 13, 000 extant species characterized [27]. Ant venoms are a complex mixture of molecules, including salts, sugars, formic acid, biogenic amines, alkaloids, free amino acids, hydrocarbons, peptides and proteins [28-31], and they play an important role in defense their nest against predators, microbial pathogens, ant competitors, and to hunt prey. The bioactivities of ant venom mainly include paralytic, cytolytic, haemolytic, antimicrobial activity, allergenic, pro-inflammatory, and insecticidal activity and others. Due to the small size of ants and the limited amount of venom produced by each ant, many ant venoms remain unexplored. Even so, the studies have revealed that the ant venoms are rich in peptides [32,33]. To date, 75 peptide toxins have fully been sequenced [34], and their bioactivity involved antimicrobial, insecticidal, haemolytic, cytolytic, paralytic activity.

Peptide toxins in ant venom play an important role in inhibiting antimicrobial infections. Ponericins from the Venom of the ant *Pachycondyla goeldii* [35] showed not only antibacterial activity against both Gram-positive and –negative bacterial, but also antifungal activity. The primary sequence of ponericins showed similarities to other peptide toxin derived AMPs. Another group of peptides toxins from the ant venom are pilosulins. Pilosulin 1 and pilosulin 2 were isolated from the venom of an Australian ant species of the *Myrmecia pilosula* species complex. Their cDNA clone derived peptides pilosulin 3 and pilosulin 4 showed potent antibacterial activities against both gram positive and Gram negative bacteria, but no antifungal activity against *L. garvieae*, *C. albicans*, and *S. cerevisiae* [36]. Bicarinalin were recently isolated as the most abundant peptide from the venom of the ant *Tetramorium bicarinatum*. Bicarinalin was active against fifteen microorganisms with minimal inhibitory concentrations. *Cronobacter sakazakii*, *Salmonella enterica*, *Candida albicans*, *Aspergilus niger* and *Saccharomyces cerevisiae* were particularly susceptible to it [37].

Wasp peptide toxins: More than 20, 000 solitary wasps are inhabited on the planet and their venoms were used for defending themselves and their colonies from the attacks by their enemies and predators. However, only a few solitary wasp venoms were chemically studied. The wasp venoms were mainly comprised of biogenic amines, peptides and proteins, which act together to induce local pain and damage, even death in large animal and human. The wasp venoms have been investigated mostly for their neurotoxins acting on different ion channels, receptors and nervous systems. Recently, α-helical cytotoxic peptide toxins with antimicrobial activity have been discovered in wasp venom, such as mastoparan, polybia-MPI, polybia-CP, protonectin, eumenitin, anoplin.

Mastoparan was initially isolated from the hornet venom and later mastoparan and some mastoparan-like peptides were isolated from a variety of social wasp venoms [38]. Mastoparan and most of the mastoparan-like peptides are mast cell degranulating peptide. They are comprised of both hydrophobic and basic amino acid and with an amphipathic chemical character. When their secondary conformation were determined, membrane mimicking environment like SDS (sodium dodecyl sulfate) and TFE (trifluoroethanol) by CD (circular dichroism), they takes a typical α-helical conformation, which was typical conformation of AMPs and was important for their antibacterial activity [39]. Polybia-MPI and polybia-CP also are two kind of mastoparan-like peptides, isolated from the venom of social wasp Polybia paulista. Both of them showed potent antibacterial activity against Gram-positive and Gram-negative bacteria, antifungal activity against laboratory standard fungi strains and clinic isolated fungi [40-43]. Protonectin was isolated from the venom of neotropical social wasp Agelaia pallipes with twelve amino acids. Protonectin could inhibit the growth of both bacteria and fungi [44,45]. Other mastoparan-like peptides such as EMP-AF, EMP-OD (isolated from the venom of Eumenine solitary wasps) also showed the same biological activities as mastoparan [46,47]. The ultrashort peptide toxin isolated from wasp is Anoplin, which has only 10 amino acids. However both its biological feature and chemical feature was similar as mastoparan-like peptides [48]

Bee peptide toxins: Bees are a monophyletic lineage within the super family Apoidea, which are currently considered as a clade Anthophila. There are nearly 20,000 known species, which are found on every continent except Antarctica. Bee venom has been used as a therapeutic agent for alleviation of pain, inflammation, and some immune system-related diseases such as rheumatoid arthritis and multiple sclerosis [49]. Bee venom was comprised of enzymes, biogenic amines, protease inhibitors, and several biologically active peptides/proteins such as melittin, adiapin, apamine, bradykinin, cardiopep, mast cell degranulating peptide, phospholipase A2 and secapin.

Melittin was the principal component of the water-soluble phase of honey bee venom. It has been reported to have inhibitory effects on microbes, such as bacteria and virus [50,51]. Secapin is one of agents in bee venom that have a significant role in therapy. First of all, secapin derived from the venom of *Apis mellifera carnica* (Ac-secapin) was demonstrated to exhibit anti-bacterial activity [52]. Then secapin-1 (AcSecapin-1) isolated from the venom of Asiatic Honey bee Ap iscerana was found that it could bind to bacterial and fungal surfaces and exhibited anti-microbial activity against fungi and gram-positive and gram-negative bacteria [53]. HYL was a peptide toxin that was isolated from the Wild Bee *Hylaeus signatus* Venom. It exhibited weak antimicrobial activity against several strains of pathogenic bacteria and moderate activity against *Candida albicans* [54]. AcICK (isolated from the venom of bee *Apis cerana*) was an ICK peptides derived from *Apis cerana* acting as an antifungal peptide [55].

Centipede peptide toxins: Centipedes are one of the oldest

extant terrestrial arthropods. There are about 3,300 species of centipidede [56]. However, the venoms of centipede are less explored till now. Peptide toxins in the known centipede venoms exhibit broad bioactivity, including ion channel modulating, antimicrobial, enzymatic, anticancer activity. At present, a few types of peptide toxins with antimicrobial activity have been reported.

Scolopin 1, scolopin 2 were 2 of the reported peptide toxins with antimicrobial activity in centipede venom. They were isolated from the venom of centipede *S. subspinipes mutilans*. Both of them showed potent antimicrobial activity against bacteria and fungi [57,58]. Other peptide toxins derived from the whole body of centipede include scolopendin 1, scolopendin 2, scolopendrasin I, scolopendrasin II, scolopendrasin VII and new lactoferricin B-like peptides. Scolopendin 1 and scolopendin 2 were identified by choi et al and Lee et al respectively from centipede *Scolopendra subspinipes mutilans.* They showed potent antimicrobial activity against several strains of bacteria and fungi [59,60]. Scolopendrasin I, scolopendrasin II, scolopendrasin VII also were derived from centipede Scolopendra subspinipes mutilans. These three peptide toxins were reported to have potential antimicrobial activity against bacteria and fungi [61-63]. In addition, ten synthetic peptides derived from centipede *Scolopendra subspinipes mutilans* was also shown to have broad antibacterial activity by Yoo et al. [64].

Mechanism of antimicrobial action of peptide toxins

Due to the biopharmaceutical and structural diversity, peptide toxins have therapeutic potential. Peptide toxins generally target at a wide variety of membrane bound ion channels, transporters, receptors, enzymes and other, which are associated with the cellular signaling pathways. Peptide toxin could affect the physiological activity of the cell by inhibiting or activating the ion channels, transporters, enzymes, receptors and exert corresponding bioactivity/ pharmaceutical activities against pain, tumor, diabetes, coronary syndrome.

With the advantages of the development of miniaturized bioassays and the improvement of mass spectroscopy, many peptide toxins were isolated and identified. Among them, a variety of peptide toxins with broad spectrum antimicrobial activity such as antibacterial, antifungal, antiviral and anti-antiprotozoal activity were given consideration by scientists, who believed that peptide toxins were likely to alleviate pathogens' resistance toward standard medication. Most of peptide toxins with antimicrobial activity are cytolytic, which is able to break the integrity of cell membrane, resulting in the leakage of cell content and even cell death. Some peptide toxins could discriminate the membrane of microbes and human cell, with selectivity toward pathogens and host cells. As we know, both bacteria and fungi have cytoplasma membrane surrounding the cell. The bacterial membranes are comprised of negative phospholipids, with lipoteichoic acids (LTAs) embedded in Gram-positive bacterial membrane and lipopolysaccharide (LPS) embedded in Gram-negative bacterial membrane [65]. For fungal cells, the selectivity could be attributed to the difference of main neutral lipid component in fungi and mammalian cells. Ergosterol is the main neutral lipid component and most important for the life of fungi, while in mammalian cells the main neutral lipid was cholesterol. In addition, the polysaccharides in the cell wall of fungal cells also involved in the selectivity, since they could binding with peptides [42]. The cationic peptide toxins derived AMPs bind with the pathogen membrane through electrostatic forces, then alter the phospholipid in the membrane and finally lead to the change of permeability. At present, there are mainly two intended mechanistic models planned to interpret the cell membrane disruption: the carpet model, in which AMPs act in a detergent like manner, covering the cell surface until a threshold concentration is reached that leads to the formation of membrane patches in which the lipids form toroidal aggregates stabilized by the amphipathic peptides; (2) The Barrel–Stave model, in which bundles of amphipathic helices oligomerize and form transmembrane pores with the hydrophilic residues facing the lumen of the pore [66]. However, for the biological membrane is highly dynamic supra molecular complexes and the relevant liquid–crystalline state of the system is disordered, it is difficult for experimental method to obtain model membrane or explain the detailed mechanism of peptide–membrane interaction. Molecular dynamics (MD) simulations offers an effective approach for developing a dynamical view of various molecular processes at an atomic level and providing a useful framework to interpret experimental results [67]. MD simulations of peptides in atomistic bilayer models allow atomic-resolution studies of peptide and lipids, and modeling of their dynamic properties and functional mechanism [68]. Furthermore, peptide toxins acted as membrane-permeated agents in the venom also could facilitating the passage of other venom toxin through the cellular barriers to their cellular targets.

In addition to direct membrane lytic action mode, peptide toxins also could affect the activity of other targets to exert their antimicrobial activity. Prabutoporin and opistoporins could interact with coupled G proteins and affect the calcium signaling and inhibit the growth of bacteria [69]. Some other peptide toxin could bind with microbiology related definite gated ions channels [70]. Hetru C [71] showed that androctonin, isolated from the scorpion could induce a significant decrease of oxygen consumption and adenosine triphosphate generation and finally affect the intracellular energetic machinery. ROS generated as a natural byproduct of the normal metabolism of oxygen and had important roles in cell signaling and maintaining internal homeostasis [26]. However, under the environmental stress, ROS levels could increase dramatically, resulting in significant damage to cell structures. Recent studies demonstrate that peptide toxin could induce the endogenous ROS to exert its antifungal effect [45]. In addition, the Imperatoxin-1, isolated from the venom of pandinum imperator scorpion, exhibits a phospholipase A2 activity and inhibits sorts of human protozoal infection [72].

Structure of peptide toxins

Research of peptide structures are mainly determination of amino acid composition and sequence in the peptides. For short peptides, elemental analysis, infrared spectroscopy(IR), nuclear magnetic resonance spectroscopy (NMR), amino acid composition analysis, mass spectrometry (MS) and other common methods, and aresufficient to explain its structural features. However, in the study of the structure of the middle and long peptides, the information available for analysis, such as UV, IR, and NMR, is limited. Using a variety of MS techniques, it provides information on the molecular weight and sequence of the peptide, which is a good complement to the Edman degradation. Now, the Unity-Inova Superconducting Nuclear Magnetic Resonance Spectrometer (Unity-Inova SNMR) is used to monitor the structure of most organic materials labeled with nuclides 1H, 13C, 15N, 17O, 19F and 31P. One-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) structures of almost all organic compounds, including the structural identification and structural confirmation of organic synthetic drugs and natural products. It is also an important approach for the study of polypeptide macromolecules.

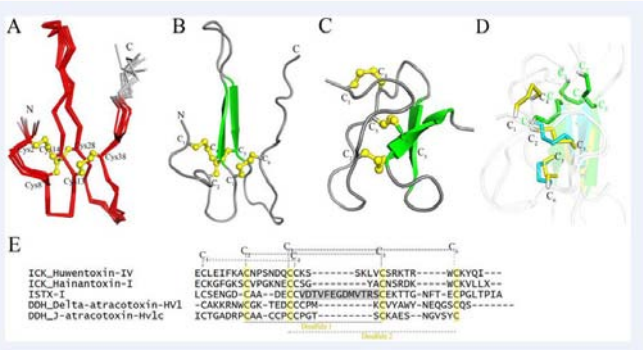

Figure 1: Three-dimensional structure of ISTX-I, with the yellow ball-and-stick format representing the disulfide bond connectivity (C 1 -C 4, C 2 -C 5 and C 3 -C 6 [80].

The biologically active peptide toxins with diverse pharmacological function have high potency and selectivity for a range of targets. The biodiversity could be attributed to the diversity of structure, which makes peptide toxin a potential source of leads and structural templates for drug development. Based on the structure, peptide toxins could be classified into linear peptides, dimeric peptides and inhibitor cysteine knot (ICK)-like peptides.

Most of the reported peptide toxins were small, cationic linear peptides, which takes a α-helix, β-sheet, and random coil conformation in the membrane environment. The conformation is very important for exhibiting the activity of peptides. These peptides demonstrate broad spectrum antibacterial, antifungal activity. Dimeric peptides are peptides with two subunit covalently linked with disulfide bond [73]. Dimeric peptides are rare in animal venom. Only a few of them were reported in scorpion, spider and marine cone snails' venom. Dimeric peptides mainly are neurotoxic peptides. Only a few of them showed antimicrobial activity. For instance, Pilosulin 3, isolated from the venom of *Myrmecia pilosula*, displays antibacterial activity [36]. Dimerization was also demonstrated to be a potential way to increase the activity of certain venoms [74]. The inhibitor cysteine knot (ICK) motif was defined as an embedded ring formed by disulfide bonds Cys (I-IV) and Cys (II-V) and their connecting backbone segments through which is threaded a third disulfide bond Cys (III-VI) , forming a cysteine knot [75]. It is invariably associated with a nearby anti-parallel β-sheet and appears to be a highly effective motif for stabilizing peptide structure. There are a lot of ICK peptide toxins found in the venom of spiders, scorpions [76], ants [77] and bees [78]. The ICK motif in the peptides was stable against the digestion of protease and attracts much attention in drug design [79]. Till now, the activity of ICK peptide toxins was not fully addressed, only one ICK peptide toxin with antimicrobial activity was reported [55]. In 2016, Rong et al. [80] determined the solution structure of the peptide using 2D 1H NMR spectroscopy. In addition to the 1H-NMR-derived information, they studied three-dimensional structure of ISTX-I, with the yellow ball-and-stick format representing the disulfide bond connectivity (C 1 -C 4, C 2 -C 5, and C 3 -C 6). (Figure 1A-E) and confirmed the key disulfide bridges described previously, between Cys-2 and Cys-14, Cys-8 and Cys-28, and Cys-13 and Cys-38. The N- and C-terminal loops are primarily stabilized by the formation of disulfide bonds.

The pharmaceutical value of peptide toxins

Peptide toxins present a promising biodiversity at molecular level; they have become a natural resource for international competition and mining. The studies of the peptide toxins have important scientific significance for the analysis of the structure and function of membrane channels and receptors and the pathogenesis of human major diseases. Certain peptide toxins can be used for drug innovation as a lead for treatment of human diseases such as cardiovascular disease, diabetes, cancer and others.

Marine organisms contain a large number of novel structures, biological activity and unique biological activity of substances and their genes, which make marine organisms with unique functional and medicinal potential. Marine toxins of many species, with peculiar structure and wide range of pharmacological activities, are widely distributed. Among them, the polypeptide protein toxin directly encoded by the gene is the most toxic of natural marine biological toxins. A large number of active peptide toxins have been isolated from marine organisms, such as representative marine peptide toxins including conotoxin, sea-snake toxins, sea-anemone toxins, jellyfish toxins and sea-urchin toxins.

In the pharmaceutical research and development, the potential of peptide toxins have been recognized by pharmaceutical researchers. In this review, we focus on peptide toxins with antimicrobial activity found in the venom of spiders, snakes, wasps, bees, scorpions, ants and centipedes (Table 2). These peptide toxins have strong pharmacological activity and small dosage. Therefore, the protein toxins can provide guidance for development of new drugs in the future.

Conclusion and Prospects

The purpose of this review is to introduce the possible use of animal venom as potential source of alternatives in the treatment of infectious disease. Due to the biodiversity and structural diversity of biomolecules venom become a promising source of naturally occuring bioactive compounds. Among all the complex components in the animal venom, peptide toxins in the venom play an important role in exerting their therapeutic function.

Most of the reported peptide toxins from animal venom are linear and amphipathic with hydrophobic and basic amino acid in their sequence. These peptides target at plasma membrane and exert their antimicrobial activity by disrupting the integrity of the membrane. Since the component and structure of membrane usually are very conserved, it is rare for microbes to change the membrane to avoid attack under the selective pressure. So it was difficult for microbes to develop resistance and was therefore believed to be one of the priorities of peptide toxins to be developed as novel antimicrobial agents. There are also a few peptide toxins containing disulfide bond in their sequence, which exert their antimicrobial activity by blocking the microbe involved ion channels and other targets.

Although peptide toxins are ideal alternatives to conventional antibiotics, for therapeutic application, there are still some obstacles, such as high manufacture cost, susceptibility to protease, systematic safety, and hemolyis. In addition, studies of the pharmacokinetics and optimized delivery to action sites of peptide toxins have not been performed. The bioactivities and precise mechanism of action of peptide toxins remain unclear and addressed for their therapeutic potential. As we know, animal venoms are rich in peptide toxins. However, a small fraction of them has been identified and studied to date. With the identification of more and more peptide toxins, there is no doubt that venoms will be a huge pharmacological library of antimicrobial peptides. A large number of peptide toxins will be

Table 2: Some Peptide toxins with antimicrobial activity isolated from venomous animals.

Sources	Peptide toxins	Species	Antimicrobial activity	Ref.
Scorpions	Androtonin	*Androctonus australis*	Antibacterial/antifungal	[4]
	Parbutoporin	*Parabuthus schlechteri*	Antibacterial	[6]
	Opistoporins	*Opistophthalmus carinatus*	Antibacterial	[6]
	TstH	*Tityus stigmurus*	Antifungal	[8]
	Imperatoxin-I	*Pandinus imprerator*	Anti-protozal	[7]
	vpAmp 1.0	*Vaejovis punctatus*	Antibacterial/antifungal	[10]
	vpAmp 1.0	*Vaejovis punctatus*	Antibacterial/antifungal	[10]
	opisin	*Opistophthalmus glabrifrons*	Antibacterial	[11]
	Stigmurin	*Tityus stigmurus*	Antibacterial/antifungal	[12]
	Ctriporin	*Chaerilus tricostatus*	Antibacterial	[13]
	Hp1404	*Heterometrus petersii*	Antibacterial	[14]
Snakes	Cardiotoxin 3	*N. naja atra*	Antibacterial	[15]
	Tonxin γ	*Naja nigricollis*	Antibacterial	[16]
	Crotamine	South American rattlesnake	Antibacterial/antifungal/antileishmanial	[17-19]
Spiders	Lycotoxin I	*Lycosa carolinensis*	Antibacterial	[20]
	Lycotoxin II	*Lycosa carolinensis*	Antibacterial	[20]
	Latarcins	*Lachesana tarabaevi*	Antibacterial/antifungal	[21]
	Oxyopinins	*Oxyopes kitabensis*	Antibacterial	[22]
	Cupiennin 1a	*Cupiennius salei*	Antibacterial	[23]
	LyeTx I	*Lycosa erythrognatha*	Antibacterial/antifungal	[24]
	Ep-AMP1	*Echinopsis pachanoi*	Antibacterial	[25]
	Lycosin-II	*Lycosa singoriensis*	antibacterial	[26]
Ants	Ponericins	*Pachycondyla goeldii*	Antibacterial/antifungal	[35]
	Pilosulins	*Myrmecia pilosula*	Antibacterial	[36]
	Bicarinalin	*Tetramorium bicarinatum*	Antibacterial/antifungal	[37]
Wasps	Mastoparan	hornet venom/ social wasps	Antibacterial	[39]
	Polybia-MPI	*Polybia paulista*	Antibacterial/antifungal	[40-43]
	Polybia-CP	*Polybia paulista*	Antibacterial/antifungal	[40-43]
	Protonectin	*Agelaia pallipes*	Antibacterial/antifungal	[44,45]
	EMP-AF	*Eumenine solitary wasps*	Antibacterial	[46,47]
	EMP-OD	*Eumenine solitary wasps*	Antibacterial	[46,47]
	Anoplin	*Anoplius samariensis*	antibacterial	[48]
Bees	Melittin	Honey bees	Antibacterial/antifungal	[50,51]
	AcSecapin-1	*Apis mellifera carnica*	Antiviral	[52,53]
	HYL	*Hylaeus signatus*	Antibacterial/antifungal	[54]
	AcICK	*Apis cerana*	Antibacterial/antifungal	[55]
Centipedes	Scolopin 1	*S. subspinipes mutilans*	Antibacterial/antifungal	[57,58]
	Scolopin 2	*S. subspinipes mutilans*	Antibacterial/antifungal	[57,58]
	scolopendin 1	*S. subspinipes mutilans*	Antibacterial/antifungal	[59,60]
	scolopendin 2	*S. subspinipes mutilans*	Antibacterial/antifungal	[59,60]
	scolopendin 1	*S. subspinipes mutilans*	Antibacterial/antifungal	[61-63]
	scolopendin 1	*S. subspinipes mutilans*	Antibacterial/antifungal	[61-63]

explored as therapeutics or act as templates for drug design.

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