Chapter

Scorpion Toxins from *Buthus martensii* Karsch (BmK) as Potential Therapeutic Agents for Neurological Disorders: State of the Art and Beyond

Xiaoli Wang, Shuzhang Zhang, Yudan Zhu, Zhiping Zhang, Mengyao Sun, Jiwei Cheng, Qian Xiao, Guoyi Li and Jie Tao

Abstract

Scorpions are fascinating creatures which became residents of the planet well before human beings dwelled on Earth. Scorpions are always considered as a figure of fear, causing notable pain or mortality throughout the world. Their venoms are cocktails of bioactive molecules, called toxins, which are responsible for their toxicity. Fortunately, medical researchers have turned the life-threatening toxins into life-saving therapeutics. From Song Dynasty in ancient China, scorpions and their venoms have been applied in traditional medicine for treating neurological disorders, such as pain, stroke, and epilepsy. Neurotoxins purified from Chinese scorpion *Buthus Martensii* Karsch (BmK) are considered as the main active ingredients, which act on membrane ion channels. Long-chain toxins of BmK, composed of 58–76 amino acids, could specifically recognize voltage-gated sodium channels (VGSCs). Short-chain BmK toxins, containing 28–40 amino acids, are found to modulate the potassium or chloride channels. These components draw attention as useful scaffolds for drug-design in order to tackle the emerging global medical threats. In this chapter, we aim to summarize the most promising candidates that have been isolated from BmK venoms for drug development.

Keywords: scorpion toxins, BmK, neurological disorders, VGSCs, potassium channels, chloride channels

1. Introduction

Recent advances underlying medical studies have illuminated that several neurological disorders such as epilepsy, chronic pain, multiple sclerosis, stroke, brain tumor etc. are induced by dysfunction of membrane ion channels [1–3]. Up to now, multiple drugs specifically targeting ion channels have been designed to treat the diseases [4]. Some clinical studies and trials have also been initiated to discriminate therapeutic potentials of natural toxins and their derivatives such as scorpion toxins, spider toxins, snake toxins, sea anemone toxins, and toad venom, which could recognize relevant ion channels [5].

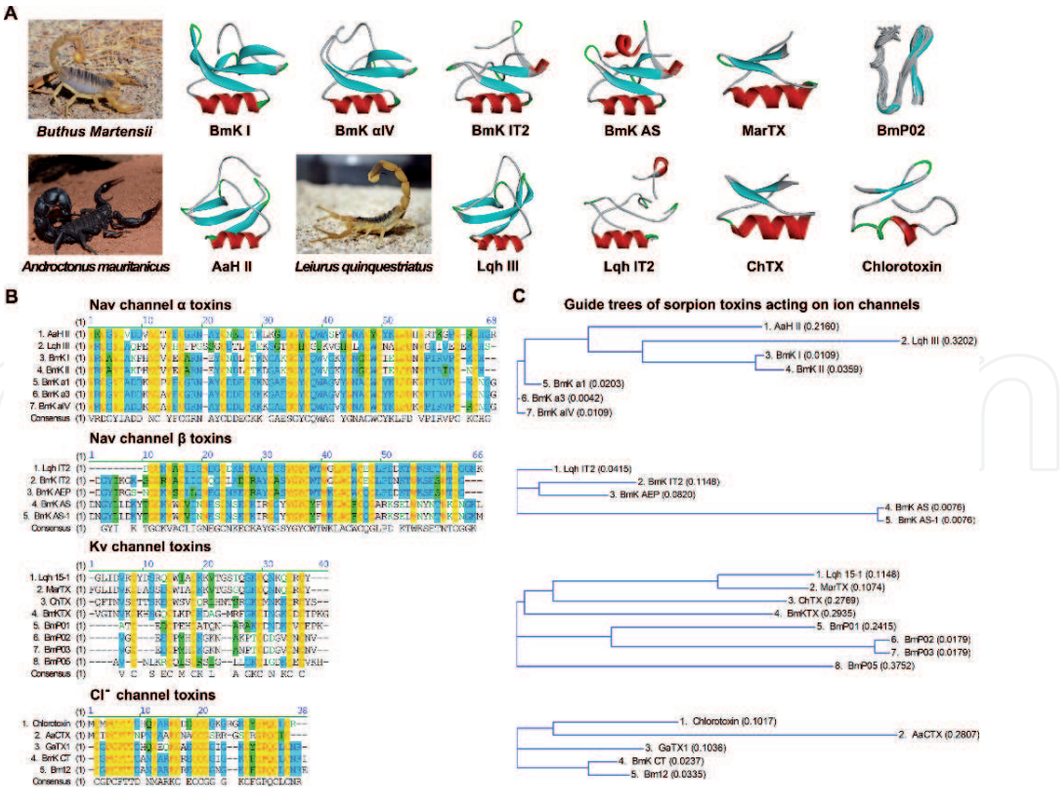

Figure 1.
Structures of scorpion toxin peptides. (A) The α/α-like scorpion toxins BmK I (PDB: 1SN1) [6] and BmK αIV [7–9] (using LQQ III, PBD: 1LQQ; BmK I, chimera Lqh αIT/AaH II, PBD: 1SEG; BmK α2, PDB: 2KBJ as templates) isolated and purified from Buthus martensii *Karsch, AaH II [10] (PDB: 1PTX) isolated and purified from* Androctonus mauritanicus*, Lqh III [11] (PDB: 1BMR) isolated and purified from* Leiurus quinquestriatus*. The β/β-like scorpion toxins BmK IT2 [7, 12–14] (using Lqh IT2, PBD: 2I61; LQQ III, PBD: 1LQQ; Lqh αIT A39L, PDB: 2YEO; Kurtoxin, PDB: 1T1T as templates), BmK AEP (using the same templates as BmK IT2), and BmK AS/AS-1 [9, 12, 14–16] (using Kurtoxin, PDB: 1T1T; Lqh IT2, PBD: 2I61; CsE-V, PBD: 1NRB; Ts3, PBD: 5CY0; BmK α2, PDB: 2KBJ as templates) isolated and purified from* Buthus martensii *Karsch. Lqh IT2 [12] (β-sheet not shown) isolated and purified from* Leiurus quinquestriatus*. The short-chain scorpion toxins acting on K^+ channels. The toxins MarTX [17] (PDB: 1M2S) and BmP02 [18] (PDB: 1DU9) isolated and purified from* Buthus martensii *Karsch. ChTX [9] (PDB: 2CRD) isolated and purified from* Leiurus quinquestriatus*. The short-chain scorpion toxins acting on Cl^- channels. The toxin chlorotoxin [19] (PDB: 2CRD) isolated and purified from* Leiurus quinquestriatus*. Sequence homology comparison is obtained by using PSI-Blast, and homology modeling of scorpion toxins is acquired by using Discovery Studio 2017 R2. (B) Top, multiple sequence alignment of α/α-like scorpion toxins. Middle, the second one in figure B, multiple sequence alignment of β/β-like scorpion toxins. Below, the third one in figure B, multiple sequence alignment of toxins acting on K^+ channels. Bottom, multiple sequence alignment of toxins acting on Cl^- channels. Conserved residues and cysteines formatting intrachain disulfide bonds are in red and shadowed in yellow; residues conserved in most of the peptides are shadowed in blue; residues with same charge in most of the peptides are shadowed in green. The species of toxins are mentioned above, except for Lqh 15–1 [20] and GaTX1 [21] isolated and purified from* Leiurus quinquestriatus *hebraeus; BmK II, BmK a1, BmK a3, BmKTX, BmP01, BmP03, BmK CT, and Bm12 isolated and purified from* Buthus martensii *Karsch [22]; AaCTX [23] isolated and purified from* Androctonus australis*. (C) The guide tree is constructed by ALIGNX, a component of the VECTOR NTI 11.0 software suite. Scores in the brackets are based on the identity of the amino acids' chemical properties. Top, the guide tree of α/α-like scorpion toxins. Middle, the guide tree of β/β-like scorpion toxins. Below, the guide tree of short-chain K^+ channel toxins. Bottom, the guide tree of short-chain Cl^- channel toxins.*

BmK scorpion, used as a drug which is also known as "Quan Xie" (whole scorpion body), can be traced to almost 2000 years ago since the Song Dynasty (A.D. 960–1279) of China. Based on the traditional Chinese medicine theories of "Xi Feng Zhi Jing, Gong Du San Jie, Tong Luo Zhi Tong" (suppressing the epileptic seizure, inhibiting growth and metastasis of tumor, dredging blood vessels and analgesia), BmK scorpion has been widely used to treat epilepsy, apoplexy, spasm, migraine, tetanus etc. [24]. The venom of BmK scorpion, considered as the main effective component, is a rich source of bioactive toxin polypeptides that regulate the activity of ion channels [25, 26] (**Figure 1**). According to the length of these

peptides, scorpion toxins are classified into long-chain toxins and short-chain toxins. The long-chain scorpion toxins composed of 58–76 amino acid residues mainly act on voltage-gated sodium channels (VGSCs), while the short-chain scorpion toxins containing 28–40 amino acid residues generally target K^+ or Cl^- channels [27] (**Figure 1B**). Based on their physiological effects on VGSC gating and binding properties, the long-chain toxins can be further classified into two categories: α-toxins, such as BmK I, a 64-residue α-like toxin isolated from BmK [2], and BmK αIV, a novel cloned 68-residue polypeptide, binding to neurotoxin receptor site 3 of the VGSC, with inhibitory effects on the fast inactivation of VGSCs (**Figure 2**). β-toxins, which bind to receptor site 4 such as BmK IT2 as well as BmK AEP, two 64-residue inhibitory β-toxins [28], and BmK AS, a 66-residue β-like toxin, could shift the threshold of VGSCs activation to more negative membrane potentials [29–32] (**Figure 2**). By sequence alignment and phylogenetic trees, it could be found that the primary structure of BmK I is similar to that of the classical α-like toxin Lqh III, while the structural properties of BmK αIV are more similar to that of the classical α-toxin AaH II (**Figure 1C**). In addition, the structure of BmK IT2 and BmK AEP are similar to that of the classical β-toxin Lqh IT2, but it is quite different from the structure of BmK AS which is also separated from BmK (**Figure 1C**). Among short-chain toxins, martentoxin and BmP02 are considered as the specific blockers of BK channel (α + β4) and Kv1.3, respectively [33–35]. From sequence alignment and phylogenetic trees, martentoxin have low homology with the classical BK channel blocker charybdotoxin (ChTX), isolated and purified from

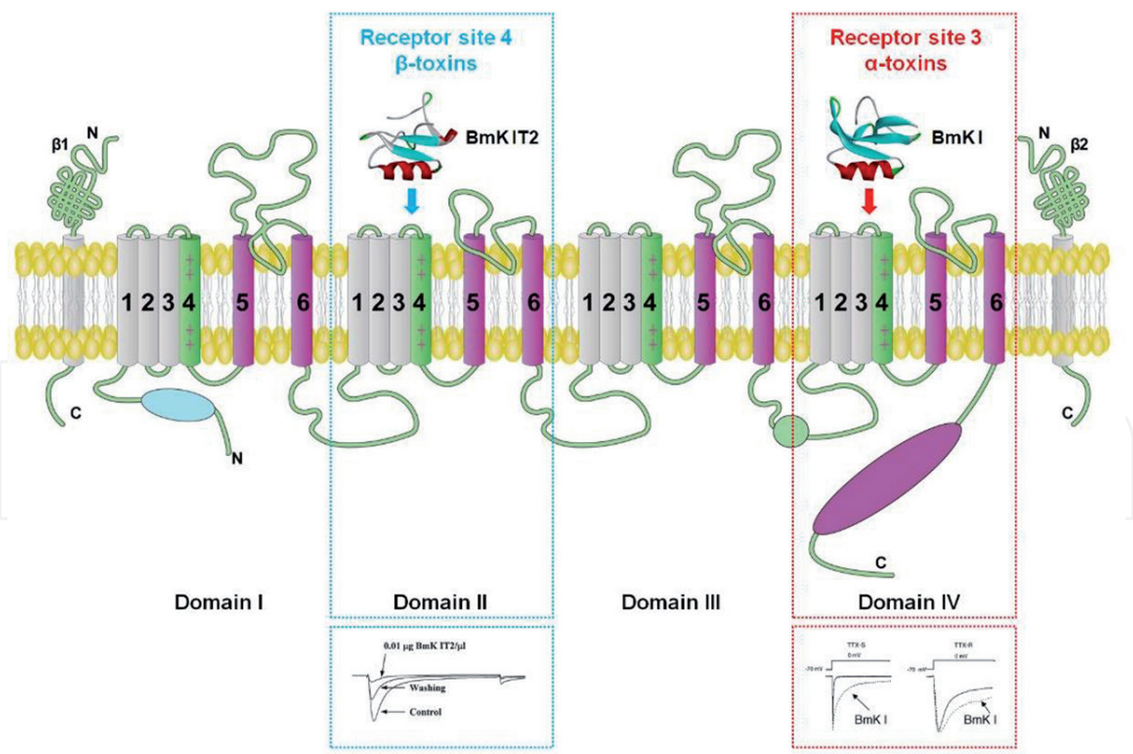

Figure 2.
Structure of VGSC and its pharmacological characterization modulated by α/α-like or β/β-like scorpion toxins. Schematic representation of Nav channels' α and β subunits. The α subunit of Nav channels is illustrated along with not only β1 but also β2 subunits; the β subunits' extracellular domains are exhibited to be an immunoglobulin-like fold that interacts with the loops in α subunits. The domains of the α subunit are represented by Roman numerals; segments 5 and 6 (exhibited in violet) are pore-lining segments, and S4 helices (green) constitute the voltage sensors. The green circle in the domains III and IV intracellular loop represents the inactivation-gating IFM motif. The α/α-like toxin BmK I delaying the inactivation of VGSCs by targeting the receptor site 3. The β toxin BmK IT2 suppressing transient currents of VGSCs by targeting the receptor site 4.

Leiurus quinquestriatus, but have high homology with Lqh 15–1 (ChTX2), another BK channel blocker from *Leiurus quinquestriatus* (**Figure 1B** and **C**). BmK CT could recognize the glioma-specific chloride channels. In this chapter, we aim to describe the most promising candidates for drug development that have been isolated from BmK venoms, with categorization according to their biological activity.

2. Analgesic effects of BmK toxins against VGSCs

Pain seriously damages human health and quality of life, so it is of importance to find effective analgesic targets and drugs. Nav channels (VGSCs) are transmembrane proteins responsible for generation and conduction of APs (action potentials) in excitable cells [1–3]. Of the nine functional α subunits (Nav1.1–1.9), Nav1.1, Nav1.3, Nav1.6, Nav1.7, Nav1.8, and Nav1.9 are distributed in primary sensory neurons, playing a crucial role in nociception and chronic pain [4]. In detail, mechanical pain is mainly caused by Nav1.6 [5]. Functional acquired mutations in Nav1.7 cause severe thermal hyperalgesia [24], while Nav1.7 with loss-of-function mutations leads to pain insensitivity [25]. Nav1.8 contributes to the APs generation in peripheral system and Nav1.9 plays a role in persistent Na+ currents in small-diameter dorsal root ganglia (DRG) neurons [26]. Because of the critical roles of VGSC subtypes in pain signal conduction [36, 37], natural products, specifically inhibiting VGSCs, might reveal the potential for treating chronic pain symptoms [38]. The neurotoxins of scorpion BmK are an excellent source of sodium channel modulators [22]. Among them, β/β-like scorpion toxins, binding to VGSC receptor site 4 such as BmK IT2 and BmK AS, show anti-nociceptive effects in *in vivo* experiments [39, 40].

BmK AS, polypeptide composed of 66 amino acid residues purified from BmK venom, was a unique β-like scorpion toxin with many distinct functions [41]. In the peripheral nervous system (PNS), BmK AS-induced antinociceptive effect on inflammation-induced thermal as well as spontaneous pain and mechanical hyperalgesia [30, 42]. Peripheral or spinal delivery of BmK AS significantly suppressed formalin-induced nociceptive behaviors and c-Fos expression in spinal cord [30, 43]. In order to clarify the mechanisms underlying antinociceptive effects of receptor site 4 toxins on VGSCs, the primary sensory neurons (dorsal root ganglion, DRG) isolated from the L4-L6 of adult rats are usually chosen for investigation. Patch clamp recording showed that BmK AS could significantly decrease excitability of small DRG neurons, by depressing the peak tetrodotoxin-resistant (TTX-R) and tetrodotoxin-sensitive (TTX-S) Na^+ currents of DRG neurons, and causing a negative shift of voltage-dependent activation [30]. Furthermore, BmK AS reduces the peak currents, facilitates steady-state activation, and inhibits slow inactivation of the Nav1.3 channels [44]. Through testing the VGSCs endogenously expressed in the DRG neuroblastoma ND7–23 cells as well as heterologously expressed Nav1.2 in *Xenopus* oocytes, it exhibited a U-shaped modulation of gating kinetics by BmK AS over a wide range of concentrations. BmK AS could suppress the peak currents, facilitate steady-state activation of VGSCs endogenously expressed in ND7–23 cells, while it did not affect the voltage-dependent activation and persistent currents of Nav1.2 [45]. These results provide a better understanding of the peripheral anti-injury sensation of BmK, which selectively inhibited the activity of Nav1.3 and DRG subtypes of VGSCs.

BmK IT2, consisting of 61 amino acid residues, contains 4 disulfide bonds, and could induce strong insect toxicity [28]. Like other depressant toxins, such as LqhIT2 [46, 47], BmK IT2 possesses two non-interacting binding sites (the high/low-affinity binding sites) on insect nerve membranes [48, 49]. But a previous

study also found that formalin-induced spontaneous pain behavior and spinal c-Fos expression could be effectively suppressed by either pre- or post-treatment with intrathecal BmK IT2 [50], which strongly implied that BmK IT2 could not only bind to insect VGSCs, but also recognize mammal VGSC subtypes. In fact, the inhibition of BmK IT2 on total Na^+ currents was observed in small DRG neurons [31]. By testing VGSC subtypes in *Xenopus* oocytes expression system, Nav1.2, Nav1.3, and Nav1.6 display insensitive property to BmK IT2, suggesting that other isoforms, especially Nav1.7–1.9, might be involved in the suppressive activity of BmK IT2 in rat pathological models [51]. The results illuminated that BmK IT2 can be developed as a novel analgesic peptide with therapeutic potential.

3. Antiepileptic activity of BmK toxins

3.1 Antiepileptic activity of BmK Na^+ channel toxins

VGSCs play a critical role in the generation and propagation of neuroexcitibilty. Genetic alterations in VGSC genes are considered to be associated with epileptogenesis. The SCN1A (Nav1.1 gene) is the most relevant VGSC gene for epilepsy in clinical tests. More than 1200 Nav1.1 mutants have been characterized to be associated with epilepsy, most of variants mutations lead to febrile seizures [52]. Nav1.2 subunits are mainly distributed in the Ranvier node and axon-initiating segment (AIS). The mutation of Nav1.2 (SCN2A) is relevant to various epilepsies, such as Dravet's syndrome (DS), benign familial neonatal seizures (BFNIS), hereditary epilepsy with febrile seizures plus (GEFS+), and other stubborn childhood epileptic encephalopathies [53]. Another VGSC subtype widely distributed in CNS is Nav1.6, which is mainly distributed to the soma and synaptic origins. Mutations of Nav1.6 could induce severe epileptic encephalopathy exhibiting autistic features, early onset seizures, intellectual disability, ataxia, or sudden unexpected death in epilepsy (SUDEP) [54]. Therefore, this evidence strongly implies that natural products inhibiting VGSCs could also have the potential for suppressing the epileptic seizure.

BmK AEP, composed of 61 residues with 4 disulfide bonds, is the first anti-epilepsy peptide purified from scorpion venom. BmK AEP was less toxic to mice and insects, while it had forceful anticonvulsant effects on epileptic rats, and is thus named as BmK anti-epilepsy peptide (BmK AEP) [55]. BmK AEP has been reported to display anti-epileptic activity in a coriaria lactone-induced epileptic model in the rat with comparable efficacy to diazepam [56]. Recent studies demonstrated that BmK AEP concentration-dependently suppresses the Na^+ currents of Nav1.3 and Nav1.6, heterologously expressed in HEK293 cells, and shifts the voltage-dependent activation to the hyperpolarized direction, with minimal effects on steady-state inactivation [32].

Through intrahippocampal injection, β scorpion toxin BmK AS produced obviously anticonvulsant activity on the pentylenetetrazol (PTZ)-induced epileptic rodents. It could not only suppress the duration and number of high-amplitude, high-frequency discharges (HAFDs) in electroencephalography (EEG), but also obviously reduce the peak Na^+ currents of hippocampal pyramidal cells [57, 58]. By contrast, BmK AS did not regulate the epileptiform EEG of pilocarpine model over the same dose range [57]. Intrahippocampal injection of BmK AS obviously reduced the increase of c-Fos expression evoked by pilocarpine, implying that neuronal hyperactivity is decreased during the epileptic state [43].

Injection of BmK IT2 at hippocampal CA1 region could dose-dependently inhibit PTZ-induced epilepsy-like behavior as well as reduce the number and duration of HAFD on PTZ-induced epileptic EEG components. Similarly, BmK IT2 significantly

prolonged the incubation period of status epilepticus (SE) onset, reduced the severity of SE, and inhibited the expression of c-fos in the hippocampus during SE of pilocarpine-induced epileptic rodents [59]. BmK IT2, which relieves epileptic symptoms, is thought to inhibit the activity of VGSC subtypes. Binding experiments showed that BmK IT2 could recognize neuronal synaptosome membranes. The patch-clamp experiment also proved that BmK IT2 can inhibit the persistent sodium current of hippocampal pyramidal neurons [59]. However, previous studies have found that BmK IT2 had no significant inhibitory effect on the peak Na^+ currents of Nav1.2, 1.3, and 1.6 heterologous-expressed in oocytes [51]. It is suggested that BmK IT2 might act on Nav1.1 or Nav1.7 in the central nervous system (CNS).

3.2 Antiepileptic activity of BmK K^+ channel toxins

BK channels, widely expressed in CNS, are voltage- and Ca^{2+}-activated K^+ channels with large conductance [60–62] (**Figure 3**). They have been shown to modulate fast afterhyperpolarization (fAHPs) and rapid spike repolarization in a number of types of neurons [63–65]. Under pathological state, it interacted the inactivation of Nav channels, with inducing neuronal spike shortening and increasing in firing rate as well as excitatory transmitter release, which could exacerbate seizure bursts [66–68].

Pentylenetetrazol (PTZ)-induced generalized tonic-clonic seizures give rise to a BK channel gain-of-function, characterized by increased BK currents as well as neuronal firing in the somatosensory cortex [69]. Interestingly, the BK channel blocker, paxilline, suppressed generalized tonic-clonic seizures in picrotoxin or

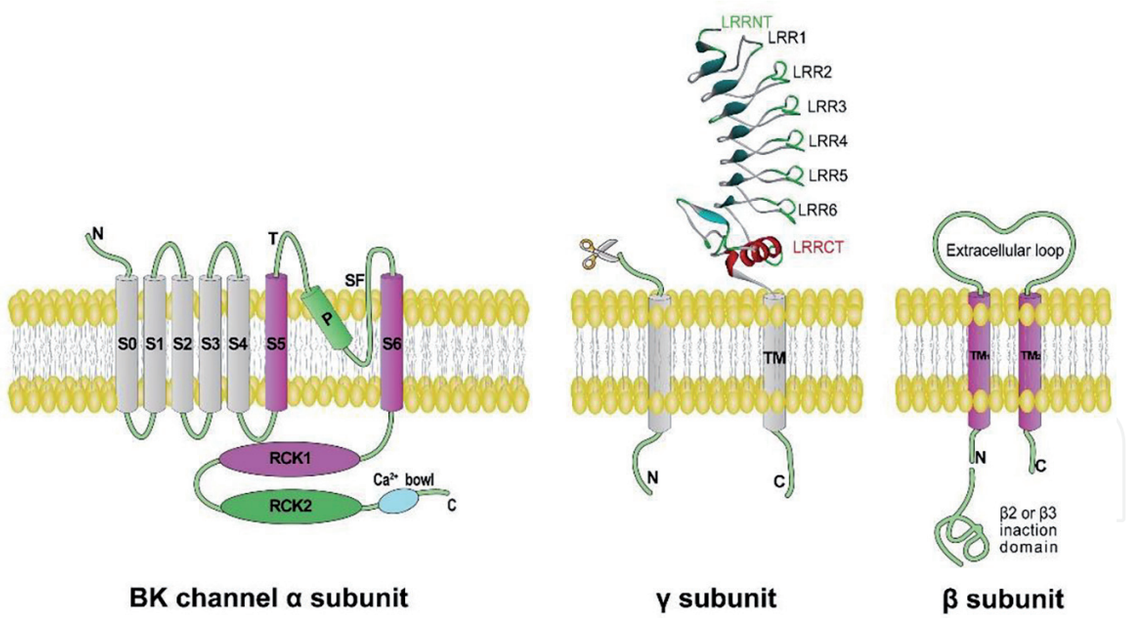

Figure 3.
Structure of BK channel and its auxiliary subunits. BK channel topology predicted by the hydrophilicity profiles (left). The α helices are represented by a cylindrical shape and S0 to S4 segments (gray column) make up the voltage sensor domain. The turret (T) is the loop joining S5 with the pore helix (P) (green column). The selective filter (SF) and S6 form the pore internal entryway. N terminal is located at the extracellular, and C terminal is located at cytosolic. Intracellular domain forms a pair of RCK domains including a Ca^{2+} bowl (light blue ellipse), one of the intracellular calcium-binding regions. Topology of auxiliary β subunits (right). NH_2 and COOH terminus facing intracellular side, two transmembrane domains linked by an extensive extracellular loop. At the NH_2 terminus, β2 and β3 subunits contain additional amino acids that constitute the particle of inactivation. The topological structure shared by all subtypes of γ subunits: after selective cleavage, the γ subunits only have one transmembrane segment, with LRRC domains and the NH2 terminus facing the extracellular side. The homology model of LRRC domain was established by using the crystal structure underlying the LRR domain from lymphocyte receptor B59 of hagfish variable (PDB ID: 2O6S) [70]. The homology modeling of LRRC domain is acquired by using Discovery Studio 2017 R2.

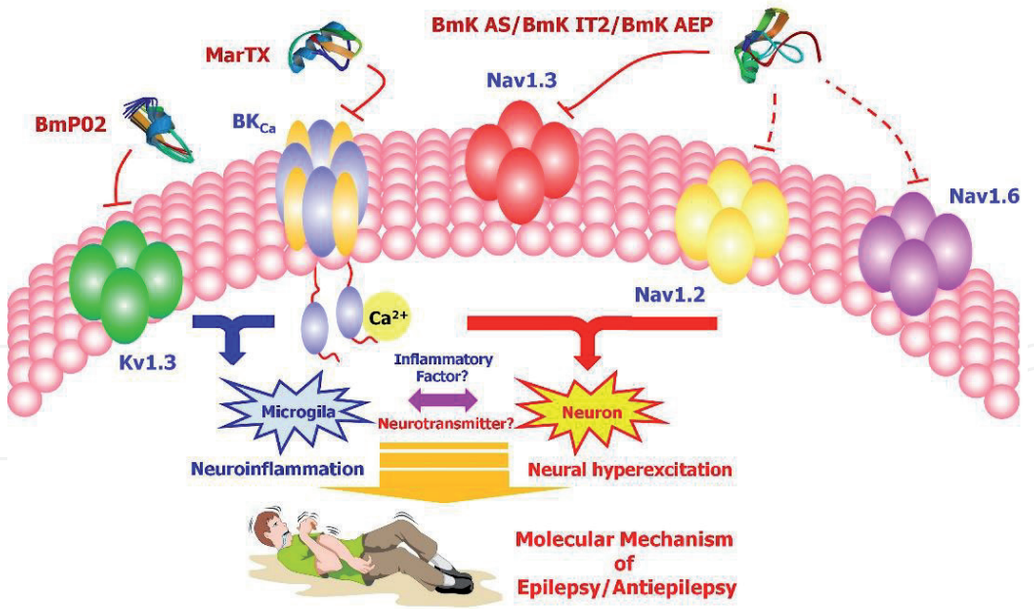

Figure 4.
Antiepilepsy effects of BmK venom toxins by targeting Na^+/K^+ channels. Long-chain β toxins BmK IT2/BmK AS/BmK AEP could reduce the epileptic seizure by inhibiting the activities of VGSCs. The specific BK channel blocker MarTX, a short-chain BmK toxin, suppressed the epilepsy by acting on the fAHPs and rapid spike repolarization of neurons, which might affect the activation of microglia through BK channels. BmP02, a Kv1.3 inhibitor, has potential antiepileptic effects also by regulating the activation of microglia.

PTZ-induced epileptic animal models, and reversed the elevated neuronal firing which follows tonic-clonic seizures [69, 71].

Martentoxin, a polypeptide consisting of 37 residues isolated from the venom of BmK, could selectively block iberiotoxin-insensitive BK channel subtye (α + β4) [33, 72], with no obvious effects on BK channels with α subunit alone. In animal model experiments, martentoxin could prolong the latency and decrease the duration, as well as seizure numbers, especially the high stage seizure, of seizures induced by PTZ. The attitude and the duration of epileptic discharge are both decreased by intra-hippocampal injection with martentoxin [73] (**Figure 4**).

4. Anti-multiple sclerosis and stroke via Kv1.3

4.1 Anti-multiple sclerosis effects of BmK Kv1.3 blockers

Multiple sclerosis (MS), a neuroinflammatory demyelinating disease, is the second most common neurological disease. The occurrence of multiple sclerosis is often accompanied by the destruction of the blood-brain barrier (BBB) and the infiltration of the central nervous system by reactive T cell [74]. These cells rapidly produce large amounts of pro-inflammatory cytokines, such as IFN-γ and IL-4, inducing sebaceous lesions or damage by targeting myelin basic proteins, thereby promoting shedding.

The potassium channel Kv1.3 was first discovered in human T-cells in 1984 [75]. Accumulated data display Kv1.3 in myelin-reactive T cells from the peripheral blood (PB) underlying MS patients is more highly expressed compared with healthy people [76]. In animal model of experimental autoimmune encephalitis (EAE), it has also been confirmed that the expression of Kv1.3 is significantly elevated [77]. Kv1.3 blocks membrane depolarization and maintains the driving force for Ca^{2+} entry by effluxing K^+, which in turn participates in T cell activation, Ca^{2+} activation

signaling cascade, leading to T cell proliferation and cytokine production [78, 79]. These findings make Kv1.3 a valuable potential therapeutic target for immunosuppression in MS and EAE [80]. The therapeutic efficacy of Kv1.3 channel blockers has been evidenced by not only in *in vitro* assays on suppressing cytokine secretion and the proliferation of T cells, but also by *in vivo* experiments on diverse animal models of autoimmune diseases [81–85].

A variety of animal toxin peptides have been found to have the same channel target. The venoms of different species such as scorpions, anemones, snakes, and cone snails constitute a peptide damper for Kv1.3 [86–88]. Studies have shown that different toxin peptides have different affinities for the Kv1.3 channel and can inhibit Kv1.3 in the picomolar to nanomolar range [89].

BmKTX is an α-KTx toxin purified from the venom of BmK with 37 amino acids, which has an amidated C-terminal, and blocks Kv1.3 current with nanomolar concentration [90, 91]. However, in addition to being selective for Kv1.3, BmKTX also has affinity for other K+ channels, which promotes the design as well as appearance of highly selective BmKTX structural analogs [92]. The BmKTX D33H variant was produced by replacing the Asp33 residue with His in BmKTX. The selectivity of this novel BmKTX analog is 10,000-fold higher than wild-type BmKTX for targeting Kv1.3 [92, 93]. ADWX-1, a novel peptide based on the scorpion toxin BmKTX, replaces three residues of BmKTX (Gly 11, Ile 28, and Asp 33) with Arg 11, Thr 28, and His 33. The ADWX-1 peptide not only has a picomolar affinity (IC 50, 1.89 pm) for blocking Kv1.3, but its activity is increased 100-fold compared to the native BmKTX toxin [94]. More importantly, ADWX-1 also showed good selectivity on Kv1.3 compared to the related Kv1.1 and Kv1.2 channels. The data show that both BmKTX-D33H and ADWX-1 can effectively inhibit the activation and subsequent proliferation of human and rat CD4 + CCR7-TEM cells and the secretion of cytokines [93, 94]. It is similar to the pharmacological properties of ShK-186, an anemone toxin analog that has been used in clinical research as a novel drug for the treatment of autoimmune diseases [95]. In addition, ADWX-1 can selectively inhibit the activation of effector memory T cells by inhibiting Kv1.3, thereby significantly improving the symptoms of experimental autoimmune encephalomyelitis (EAE) in a rat model [84, 93]. The results above illuminated that BmKTX-D33H as well as ADWX-1 have the potential for clinical treatments of Kv1.3-related channel diseases.

BmP02, also referred to α-KTx9.1, is a short peptide toxin from the BmK scorpion. It is comprised of 28 amino acids, whose tertiary structure is stabilized by 3 disulfide bonds [21, 96]. It was found that it has nanomolar affinity for Kv1.3 [35, 97]. Functional characterization of BmP02 as a highly selective and potent Kv1.3-targeted peptide will help develop novel therapeutic agents for human autoimmune diseases.

4.2 Anti-stroke potential of BmK Kv1.3 blockers

Stroke is an acute cerebrovascular disorder that causes brain tissue damage, which is the second leading disease causing sudden death after ischemic heart disease and accounts for 9% of deaths worldwide [83]. Ischemic stroke is the most common type of stroke, usually occurring when the blood vessels in the neck or brain are blocked [98]. In the early stages of stroke, activated macrophages or microglial cells (M1 type) release a variety of inflammatory factors (TNF-α, IL-1-β, IL-23), trigger neuronal damage, and induce TEM cell-mediated further inflammatory responses [99]. A few days later, macrophages could change to M2-like functions, begin to clear various inflammatory factors, cell debris, and secrete anti-inflammatory as well as neurotrophic factors (IL-10, TGF-β, IGF-1) to promote injury recovery [99].

Kv1.3 plays important roles in microglia as well as macrophage activation by modulating Ca^{2+} signaling, oxidative burst, cytokine production, and neuronal killing [100–102], which is required for microglia or macrophage M1-like pro-inflammatory activation *in vivo* [103]. Activated microglia in the pathology of ischemic stroke significantly contributes to secondary expansion of the infarct, and Kv1.3 blockers are thought to be useful in ameliorating this condition [104, 105]. Studies have shown that while Kv1.3 inhibitors preferentially inhibit "M1-like" inflammatory microglia/macrophage functions they can preserve beneficial "M2-like" functions [106, 107].

BmP02 and BmKTX act as BmK K^+ channel toxins that can effectively inhibit Kv1.3. We speculate that they and their derivatives may also reduce pro-inflammatory factors and improve brain damage by inhibiting the M1-like function of microglia or macrophages.

5. Anti-glioma activity

Glioma shows the general characteristics of tumor cells, with the difference being that the specific chloride channel current (CCC) is a unique electrophysiological feature of glioma cells. The current intensity always increases with the increase of malignant degree of glioma [108]. The specific type of chloride channel on glioma cells can regulate the morphology and volume of cells, which are involved in the process of tumor cell proliferation and metastasis. Abnormal expression of chloride channel currents in glioma could be regarded as a kind of chloride channel disease, especially in glioma with high malignancy [109]. Therefore, it may provide a novel idea for the diagnosis and treatment of glioma by blocking its specific chloride channel current, from the perspective of ion channel disease.

BmK chloride channel toxins, BmK CT, are short-chain neurotoxin proteins composed of 36 amino acids and contain 4 pairs of disulfide bonds, which have 68% homology with chlorotoxin (CTX), a chloride channel toxin isolated from scorpion *Leiurus quinquestriatus*. BmK CT could not only specifically block the glioma chloride channels, but also recognize the matrix metalloproteinases-2 (MMP-2) for inhibiting glioma migration [110, 111]. The recombinant protein GST-BmK CT significantly suppresses on tumor growth in nude mice, with an inhibition rate of 86% *in vivo*. The tumor metastasis in the lung lesion area was only 38% in the BmK CT-treated group compared to 75% in the control group [112]. In addition, BmK CT could promote the sensitivity of chemotherapeutic drug temozolomide-induced cell apoptosis of glioma U251 cells *in vitro*, which is through inhibiting the AKT signaling pathway [113]. On the one hand, the specific inhibition of the proliferation and metastasis of glioma cells suggests BmK CT as an ideal candidate to treat glioma. On the other hand, due to the abundant expression of chloride channels in glioma cells, BmK CT is also used for imaging and treating glioma by conjugating it with Cy5.5, FND, or 131I/125I [114].

6. Proposal

Up to now, there are 15 venom-derived drugs used to treat a variety of diseases, including hypertension, pain, and diabetes, in clinic. As a result, many lives have been saved. In addition, 13 animal-derived toxins are considered to be drug candidates, and have entered clinical trials [115]. Among them, scorpion toxin chlorotoxin, isolated from *Leiurus* quinquestriatus, is under phase II clinical trial. It was reported that Iodine-131-chlorotoxin (TM-601) is a targeted drug candidate for the

treatment of gliomas because it could cross the blood-brain as well as some tissue barriers and specifically bind to malignant brain tumor cells without influencing the function of normal cells [116]. ShK derivatives, ShK-186 and ShK-192, are mainly used to treat autoimmune diseases, including neuroinflammatory multiple sclerosis by targeting Kv1.3 channels. In this review, we discuss the possibility of BmK scorpion toxins for clinical treatment on ion channel-relevant neurological disorders. It is shown that long-chain scorpion toxins, such as BmK IT2 and BmK AS, could effectively suppress neuroexcitability in nociception and epileptic seizure via VGSCs. *In vivo* study demonstrated that inhibition of Kv1.3 is favorable for the reversion of neuroinflammatory diseases by BmKTX and BmP02. It is also found that BmK CT could specifically suppress proliferation as well as metastasis of glioma cells. This brings the dawn to the effective control of neurological diseases suspected of overcoming, such as chronic pain, MS, intractable epilepsy, and giloma.

However, it is still a challenge for BmK toxins used to the treatment of neurology disorders. The first problem underlying the application of these peptides is that they could not be taken orally, mainly because they are difficult to penetrate the intestinal mucosa. Due to their molecular size, polarity, hydrophilicity, and chargeability, the cell membrane penetration of BmK toxins is hampered. The second obstacle is that BmK toxins cannot cross the blood-brain barrier. Different from multiple sclerosis, the myelin and blood-brain barrier are not destroyed in other neurological diseases [117]. Clinical application of BmK toxins for treating these diseases will encounter difficulties. Fortunately, the situation is not unsolvable, we still have a glimmer of light. A few years ago, scientists at the Sunnybrook Health Science Center in Canada used focused ultrasound technology to successfully pass chemotherapy drugs across the blood-brain barrier in a non-invasive manner [118] and reach the location of the tumor, which is of great significance in the field of neuropharmacology. In addition, the cell penetrating peptide (CPP) [119] with a strong cell membrane penetration, could be used as a drug carrier to assist the passage of polypeptide drugs across the cell membrane [117]. The fusion protein consists of CPP and BmK toxin might be developed as an oral drug for treating neurological disorders. In short, finding suitable, safe, and efficient ways to promote the clinical use of BmK toxins are most valuable points to be solved.

Acknowledgements

This work was supported by National Science Foundation of China (Nos. 81603410 and 31771191); Youth Talent Promotion Project of China Association of Chinese Medicine; Shanghai Municipal Commission of Health and Family Planning Fund (Nos. 20184Y0086, 2016JP007 and 2018JQ003); Project for Capacity Promotion of Putuo District Clinical Special Disease; Project within budget of Shanghai University of Traditional Chinese Medicine (Nos. 18TS086 and 18-DX-06); the Key Speciality Program (No. 2016102A); and Research Project (Nos. 2016208A, 2018314 and 2018313) of Putuo Hospital, Shanghai University of Traditional Chinese Medicine.

Conflict of interest

The authors confirm that they have no conflict of interest with regard to this chapter's content.

DOI: http://dx.doi.org/10.5772/intechopen.90889

Author details

Xiaoli Wang[1,2], Shuzhang Zhang[2], Yudan Zhu[1], Zhiping Zhang[2], Mengyao Sun[1], Jiwei Cheng[1], Qian Xiao[1], Guoyi Li[1] and Jie Tao[1*]

1 Department of Neurology and Central Laboratory, Putuo Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China

2 Institute of Biomembrane and Biopharmaceutics, Shanghai University, Shanghai, China

*Address all correspondence to: jietao_putuo@foxmail.com

References

[1] Zhang S, Zhang Z, Shen Y, et al. SCN9A epileptic encephalopathy mutations display a gain-of-function phenotype and distinct sensitivity to oxcarbazepine. Neuroscience Bulletin. 2020;**36**(1):11-24. DOI: 10.1007/s12264-019-00413-5

[2] Zhou G, Jiao Y, Zhou Y, et al. Up-regulation of Akt and Nav1.8 in BmK I-induced pain. Neuroscience Bulletin. 2018;**34**(3):539-542. DOI: 10.1007/s12264-018-0222-x

[3] Hatta S, Sakamoto J, Horio Y. Ion channels and diseases. Medical Electron Microscopy. 2002;**35**(3):117-126. DOI: 10.1007/s007950200015

[4] Wulff H, Christophersen P. Recent developments in ion channel pharmacology. Channels (Austin, Tex.). 2015;**9**(6):335. DOI: 10.1080/19336950.2015.1077650

[5] Lewis RJ, Garcia ML. Therapeutic potential of venom peptides. Nature Reviews. Drug Discovery. 2003;**2**(10):790-802. DOI: 10.1038/nrd1197

[6] He XL, Li HM, Zeng ZH, et al. Crystal structures of two alpha-like scorpion toxins: Non-proline cis peptide bonds and implications for new binding site selectivity on the sodium channel. Journal of Molecular Biology. 1999;**292**(1):125-135. DOI: 10.1006/jmbi.1999.3036

[7] Landon C, Cornet B, Bonmatin JM, et al. 1H-NMR-derived secondary structure and the overall fold of the potent anti-mammal and anti-insect toxin III from the scorpion *Leiurus quinquestriatus* quinquestriatus. European Journal of Biochemistry. 1996;**236**(2):395-404

[8] Karbat I, Frolow F, Froy O, et al. Molecular basis of the high insecticidal potency of scorpion alpha-toxins. The Journal of Biological Chemistry. 2004;**279**(30):31679-31686. DOI: 10.1074/jbc.M402048200

[9] Zhu J, Tong X, Cao C, et al. Solution structure of BmKalphaTx11, a toxin from the venom of the Chinese scorpion *Buthus martensii* Karsch. Biochemical and Biophysical Research Communications. 2010;**391**(1):627-633. DOI: 10.1016/j.bbrc.2009.11.110

[10] Housset D, Habersetzer-Rochat C, Astier JP, et al. Crystal structure of toxin II from the scorpion *Androctonus australis* Hector refined at 1.3 Å resolution. Journal of Molecular Biology. 1994;**238**(1):88-103. DOI: 10.1006/jmbi.1994.1270

[11] Krimm I, Gilles N, Sautiere P, et al. NMR structures and activity of a novel alpha-like toxin from the scorpion *Leiurus quinquestriatus* hebraeus. Journal of Molecular Biology. 1999;**285**(4):1749-1763. DOI: 10.1006/jmbi.1998.2418

[12] Karbat I, Turkov M, Cohen L, et al. X-ray structure and mutagenesis of the scorpion depressant toxin LqhIT2 reveals key determinants crucial for activity and anti-insect selectivity. Journal of Molecular Biology. 2007;**366**(2):586-601. DOI: 10.1016/j.jmb.2006.10.085

[13] Ye P, Hua L, Jiao Y, et al. Functional up-regulation of Nav1.8 sodium channel on dorsal root ganglia neurons contributes to the induction of scorpion sting pain. Acta Biochimica et Biophysica Sinica. 2016;**48**(2):132-144. DOI: 10.1093/abbs/gmv123

[14] Ye P, Jiao Y, Li Z, et al. Scorpion toxin BmK I directly activates Nav1.8 in primary sensory neurons to induce neuronal hyperexcitability in rats. Protein and Cell. 2015;**6**(6):443-452. DOI: 10.1007/s13238-015-0154-4

[15] Jablonsky MJ, Watt DD, Krishna NR. Solution structure of an old world-like neurotoxin from the venom of the new world scorpion *Centruroides sculpturatus* Ewing. Journal of Molecular Biology. 1995;**248**(2):449-458

[16] Dang B, Kubota T, Mandal K, et al. Elucidation of the covalent and tertiary structures of biologically active Ts3 toxin. Angewandte Chemie (International Ed. in English). 2016;**55**(30):8639-8642. DOI: 10.1002/anie.201603420

[17] Wang Y, Chen X, Zhang N, et al. The solution structure of BmTx3B, a member of the scorpion toxin subfamily alpha-KTx 16. Proteins. 2005;**58**(2):489-497. DOI: 10.1002/prot.20322

[18] Xu Y, Wu J, Pei J, et al. Solution structure of BmP02, a new potassium channel blocker from the venom of the Chinese scorpion *Buthus martensi* Karsch. Biochemistry. 2000;**39**(45):13669-13675. DOI: 10.1021/bi000860s

[19] Lippens G, Najib J, Wodak SJ, et al. NMR sequential assignments and solution structure of chlorotoxin, a small scorpion toxin that blocks chloride channels. Biochemistry. 1995;**34**(1):13-21. DOI: 10.1021/bi00001a003

[20] Norton RS, Chandy KG. Venom-derived peptide inhibitors of voltage-gated potassium channels. Neuropharmacology. 2017;**127**:124-138. DOI: 10.1016/j.neuropharm.2017.07.002

[21] Fuller MD, Thompson CH, Zhang ZR, et al. State-dependent inhibition of cystic fibrosis transmembrane conductance regulator chloride channels by a novel peptide toxin. The Journal of Biological Chemistry. 2007;**282**(52):37545-37555. DOI: 10.1074/jbc.M708079200

[22] Goudet C, Chi CW, Tytgat J. An overview of toxins and genes from the venom of the Asian scorpion *Buthus martensi* Karsch. Toxicon. 2002;**40**(9):1239-1258

[23] Rjeibi I, Mabrouk K, Mosrati H, et al. Purification, synthesis and characterization of AaCtx, the first chlorotoxin-like peptide from *Androctonus australis* scorpion venom. Peptides. 2011;**32**(4):656-663. DOI: 10.1016/j.peptides.2011.01.015

[24] Zhijian C, Feng L, Yingliang W, et al. Genetic mechanisms of scorpion venom peptide diversification. Toxicon. 2006;**47**(3):348-355. DOI: 10.1016/j.toxicon.2005.11.013

[25] Deuis JR, Mueller A, Israel MR, et al. The pharmacology of voltage-gated sodium channel activators. Neuropharmacology. 2017;**127**:87-108. DOI: 10.1016/j.neuropharm.2017.04.014

[26] Housley DM, Housley GD, Liddell MJ, et al. Scorpion toxin peptide action at the ion channel subunit level. Neuropharmacology. 2017;**127**:46-78. DOI: 10.1016/j.neuropharm.2016.10.004

[27] He Y, Zou X, Li X, et al. Activation of sodium channels by alpha-scorpion toxin, BmK NT1, produced neurotoxicity in cerebellar granule cells: An association with intracellular Ca^{2+} overloading. Archives of Toxicology. 2017;**91**(2):935-948. DOI: 10.1007/s00204-016-1755-2

[28] Ji YH, Hattori H, Xu K, et al. Molecular characteristics of four new depressant insect neurotoxins purified from venom of *Buthus martensi* Karsch by HPLC. Science in China Series B, Chemistry, Life Sciences and Earth Sciences. 1994;**37**(8):955-963

[29] Cestele S, Catterall WA. Molecular mechanisms of neurotoxin action on voltage-gated sodium channels. Biochimie. 2000;**82**(9-10):883-892

[30] Chen J, Feng XH, Shi J, et al. The anti-nociceptive effect of BmK AS, a

scorpion active polypeptide, and the possible mechanism on specifically modulating voltage-gated Na^+ currents in primary afferent neurons. Peptides. 2006;**27**(9):2182-2192. DOI: 10.1016/j.peptides.2006.03.026

[31] Tan ZY, Xiao H, Mao X, et al. The inhibitory effects of BmK IT2, a scorpion neurotoxin on rat nociceptive flexion reflex and a possible mechanism for modulating voltage-gated Na^+ channels. Neuropharmacology. 2001;**40**(3):352-357. DOI: 10.1016/s0028-3908(00)00168-4

[32] Zhang F, Wu Y, Zou X, et al. BmK AEP, an anti-epileptic peptide distinctly affects the gating of brain subtypes of voltage-gated sodium channels. International Journal of Molecular Sciences. 2019;**20**(3). DOI: 10.3390/ijms20030729

[33] Tao J, Zhou ZL, Wu B, et al. Recombinant expression and functional characterization of martentoxin: A selective inhibitor for BK channel (alpha + beta4). Toxins (Basel). 2014;**6**(4):1419-1433. DOI: 10.3390/toxins6041419

[34] Wu B, Zhu Y, Shi J, et al. BmP02 atypically delays Kv4.2 inactivation: Implication for a unique interaction between scorpion toxin and potassium channel. Toxins (Basel). 2016;**8**(10). DOI: 10.3390/toxins8100280

[35] Wu B, Wu BF, Feng YJ, et al. Mapping the interaction anatomy of BmP02 on Kv1.3 channel. Scientific Reports. 2016;**6**:29431. DOI: 10.1038/srep29431

[36] Cummins TR, Sheets PL, Waxman SG. The roles of sodium channels in nociception: Implications for mechanisms of pain. Pain. 2007;**131**(3):243-257. DOI: 10.1016/j.pain.2007.07.026

[37] Ji YH, Liu T. The study of sodium channels involved in pain responses using specific modulators. Sheng Li Xue Bao. 2008;**60**(5):628-634

[38] Krafte DS, Bannon AW. Sodium channels and nociception: Recent concepts and therapeutic opportunities. Current Opinion in Pharmacology. 2008;**8**(1):50-56. DOI: 10.1016/j.coph.2007.09.007

[39] Bai ZT, Zhang XY, Ji YH. Fos expression in rat spinal cord induced by peripheral injection of BmK I, an alpha-like scorpion neurotoxin. Toxicology and Applied Pharmacology. 2003;**192**(1):78-85

[40] Zhang XY, Zhang JW, Chen B, et al. Dynamic determination and possible mechanism of amino acid transmitter release from rat spinal dorsal horn induced by the venom and a neurotoxin (BmK I) of scorpion *Buthus martensi* Karsch. Brain Research Bulletin. 2002;**58**(1):27-31. DOI: 10.1016/s0361-9230(02)00752-9

[41] Ji YH, Li YJ, Zhang JW, et al. Covalent structures of BmK AS and BmK AS-1, two novel bioactive polypeptides purified from Chinese scorpion *Buthus martensi* Karsch. Toxicon: Official Journal of the International Society on Toxinology. 1999;**37**(3):519-536

[42] Chen B, Ji Y. Antihyperalgesia effect of BmK AS, a scorpion toxin, in rat by intraplantar injection. Brain Research. 2002;**952**(2):322-326

[43] Liu T, Pang XY, Jiang F, Bai ZT, Ji YH, et al. Anti-nociceptive effects induced by intrathecal injection of BmK AS, a polypeptide from the venom of Chinese-scorpion *Buthus martensi* Karsch, in rat formalin test. Journal of Ethnopharmacology. 2008;**117**(2):332-338

[44] Liu ZR, Tao J, Dong BQ, Ding G, Cheng ZJ, He HQ, et al. Pharmacological kinetics of BmK

AS, a sodium channel site 4-specific modulator on Nav1.3. Neuroscience Bulletin. 2012;**28**(3):209-221

[45] Zhu MM, Tao J, Tan M, Yang HT, Ji YH, et al. U-shaped dose-dependent effects of BmK AS, a unique scorpion polypeptide toxin, on voltage-gated sodium channels. British Journal of Pharmacology. 2009;**158**(8):1895-1903

[46] Cohen L, Gilles N, Karbat I, Ilan N, Gordon D, Gurevitz M, et al. Direct evidence that receptor site-4 of sodium channel gating modifiers is not dipped in the phospholipid bilayer of neuronal membranes. The Journal of Biological Chemistry. 2006;**281**(30):20673-20679

[47] Gordon D, Moskowitz H, Eitan M, Warner C, Catterall WA, Zlotkin E. Localization of receptor sites for insect-selective toxins on sodium channels by site-directed antibodies. Biochemistry. 1992;**31**(33):7622-7628

[48] Li YJ, Tan ZY, Ji YH. The binding of BmK IT2, a depressant insect-selective scorpion toxin on mammal and insect sodium channels. Neuroscience Research. 2000;**38**(3):257-264

[49] Chai ZF, Bai ZT, Liu T, Pang XY, Ji YH, et al. The binding of BmK IT2 on mammal and insect sodium channels by surface plasmon resonance assay. Pharmacological Research. 2006;**54**(2):85-90

[50] Bai ZT, Liu T, Pang XY, Chai ZF, Ji YH. Suppression by intrathecal BmK IT2 on rat spontaneous pain behaviors and spinal c-Fos expression induced by formalin. Brain Research Bulletin. 2007;**73**(null):248-253

[51] He H, Liu Z, Dong B, Zhang J, Shu X, Zhou J, et al. Localization of receptor site on insect sodium channel for depressant β-toxin BmK IT2. PLoS One. 2011;**6**(1):e14510

[52] Meng H, Xu HQ, Yu L, et al. The SCN1A mutation database: Updating information and analysis of the relationships among genotype, functional alteration, and phenotype. Human Mutation. 2015;**36**(6):573-580. DOI: 10.1002/humu.22782

[53] Liao Y, Deprez L, Maljevic S, et al. Molecular correlates of age-dependent seizures in an inherited neonatal-infantile epilepsy. Brain. 2010;**133**(Pt 5):1403-1414. DOI: 10.1093/brain/awq057

[54] Veeramah KR, O'Brien JE, Meisler MH, et al. De novo pathogenic SCN8A mutation identified by whole-genome sequencing of a family quartet affected by infantile epileptic encephalopathy and SUDEP. American Journal of Human Genetics. 2012;**90**(3):502-510. DOI: 10.1016/j.ajhg.2012.01.006

[55] Zhou XH, Yang D, Zhang JH, et al. Purification and N-terminal partial sequence of anti-epilepsy peptide from venom of the scorpion *Buthus martensii* Karsch. The Biochemical Journal. 1989;**257**(2):509-517. DOI: 10.1042/bj2570509

[56] Wang CG, He XL, Shao F, et al. Molecular characterization of an anti-epilepsy peptide from the scorpion *Buthus martensi* Karsch. European Journal of Biochemistry. 2001;**268**(8):2480-2485. DOI: 10.1046/j.1432-1327.2001.02132.x

[57] Zhao R, Weng CC, Feng Q, et al. Anticonvulsant activity of BmK AS, a sodium channel site 4-specific modulator. Epilepsy and Behavior. 2011;**20**(2):267-276. DOI: 10.1016/j.yebeh.2010.12.006

[58] Liu ZR, Zhang H, Wu JQ, et al. PKA phosphorylation reshapes the pharmacological kinetics of BmK AS, a unique site-4 sodium channel-specific modulator. Scientific Reports. 2014;**4**:3721. DOI: 10.1038/srep03721

[59] Zhao R, Zhang XY, Yang J, et al. Anticonvulsant effect of BmK IT2, a sodium channel-specific neurotoxin, in rat models of epilepsy. British Journal of Pharmacology. 2008;**154**(5):1116-1124. DOI: 10.1038/bjp.2008.156

[60] Blatz AL, Magleby KL. Ion conductance and selectivity of single calcium-activated potassium channels in cultured rat muscle. The Journal of General Physiology. 1984;**84**(1):1-23. DOI: 10.1085/jgp.84.1.1

[61] Marty A. The physiological role of calcium-dependent channels. Trends in Neurosciences. 1989;**12**(11):420-424

[62] Sah P, McLachlan EM. Potassium currents contributing to action potential repolarization and the afterhyperpolarization in rat vagal motoneurons. Journal of Neurophysiology. 1992;**68**(5):1834-1841. DOI: 10.1152/jn.1992.68.5.1834

[63] Adams PR, Constanti A, Brown DA, et al. Intracellular Ca^{2+} activates a fast voltage-sensitive K^+ current in vertebrate sympathetic neurones. Nature. 1982;**296**(5859):746-749. DOI: 10.1038/296746a0

[64] Storm JF. Action potential repolarization and a fast after-hyperpolarization in rat hippocampal pyramidal cells. The Journal of Physiology. 1987;**385**:733-759. DOI: 10.1113/jphysiol.1987.sp016517

[65] Lancaster B, Nicoll RA. Properties of two calcium-activated hyperpolarizations in rat hippocampal neurones. The Journal of Physiology. 1987;**389**:187-203. DOI: 10.1113/jphysiol.1987.sp016653

[66] Quirk JC, Reinhart PH. Identification of a novel tetramerization domain in large conductance K(ca) channels. Neuron. 2001;**32**(1):13-23

[67] Tseng-Crank J, Foster CD, Krause JD, et al. Cloning, expression, and distribution of functionally distinct Ca^{2+}-activated K^+ channel isoforms from human brain. Neuron. 1994;**13**(6):1315-1330

[68] Tian L, Duncan RR, Hammond MS, et al. Alternative splicing switches potassium channel sensitivity to protein phosphorylation. The Journal of Biological Chemistry. 2001;**276**(11):7717-7720. DOI: 10.1074/jbc.C000741200

[69] Amberg GC, Santana LF. Downregulation of the BK channel beta1 subunit in genetic hypertension. Circulation Research. 2003;**93**(10):965-971. DOI: 10.1161/01.res.0000100068.43006.36

[70] Kim HM, Oh SC, Lim KJ, et al. Structural diversity of the hagfish variable lymphocyte receptors. The Journal of Biological Chemistry. 2007;**282**(9):6726-6732. DOI: 10.1074/jbc.M608471200

[71] Watanabe I, Zhu J, Sutachan JJ, et al. The glycosylation state of Kv1.2 potassium channels affects trafficking, gating, and simulated action potentials. Brain Research. 2007;**1144**:1-18. DOI: 10.1016/j.brainres.2007.01.092

[72] Shi J, He HQ, Zhao R, et al. Inhibition of martentoxin on neuronal BK channel subtype (alpha+beta4): Implications for a novel interaction model. Biophysical Journal. 2008;**94**(9):3706-3713. DOI: 10.1529/biophysj.107.122150

[73] Zhu Y, Zhang S, Feng Y, et al. The Yin and Yang of BK channels in epilepsy. CNS and Neurological Disorders Drug Targets. 2018;**17**(4):272-279. DOI: 10.2174/1871527317666180213142403

[74] Huang J, Han S, Sun Q, et al. Kv1.3 channel blocker (ImKTx88) maintains blood-brain barrier in experimental autoimmune encephalomyelitis. Cell and Bioscience. 2017;**7**:31. DOI: 10.1186/s13578-017-0158-2

[75] DeCoursey TE, Chandy KG, Gupta S, et al. Voltage-gated K^+ channels in human T lymphocytes: A role in mitogenesis? Nature. 1984;**307**(5950):465-468. DOI: 10.1038/307465a0

[76] Wulff H, Calabresi PA, Allie R, et al. The voltage-gated Kv1.3 K^+ channel in effector memory T cells as new target for MS. The Journal of Clinical Investigation. 2003;**111**(11):1703-1713. DOI: 10.1172/jci16921

[77] Rus H, Pardo CA, Hu L, et al. The voltage-gated potassium channel Kv1.3 is highly expressed on inflammatory infiltrates in multiple sclerosis brain. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**(31):11094-11099. DOI: 10.1073/pnas.0501770102

[78] Bozic I, Tesovic K, Laketa D, et al. Voltage gated potassium channel Kv1.3 is upregulated on activated astrocytes in experimental autoimmune encephalomyelitis. Neurochemical Research. 2018;**43**(5):1020-1034. DOI: 10.1007/s11064-018-2509-8

[79] Wulff H, Castle NA, Pardo LA. Voltage-gated potassium channels as therapeutic targets. Nature Reviews. Drug Discovery. 2009;**8**(12):982-1001. DOI: 10.1038/nrd2983

[80] Beeton C, Wulff H, Standifer NE, et al. Kv1.3 channels are a therapeutic target for T cell-mediated autoimmune diseases. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(46):17414-17419. DOI: 10.1073/pnas.0605136103

[81] Tudor JE, Pallaghy PK, Pennington MW, et al. Solution structure of ShK toxin, a novel potassium channel inhibitor from a sea anemone. Nature Structural Biology. 1996;**3**(4):317-320

[82] Kalman K, Pennington MW, Lanigan MD, et al. ShK-Dap22, a potent Kv1.3-specific immunosuppressive polypeptide. The Journal of Biological Chemistry. 1998;**273**(49):32697-32707. DOI: 10.1074/jbc.273.49.32697

[83] Schmitz A, Sankaranarayanan A, Azam P, et al. Design of PAP-1, a selective small molecule Kv1.3 blocker, for the suppression of effector memory T cells in autoimmune diseases. Molecular Pharmacology. 2005;**68**(5):1254-1270. DOI: 10.1124/mol.105.015669

[84] Li Z, Liu WH, Han S, et al. Selective inhibition of $CCR7^-$ effector memory T cell activation by a novel peptide targeting Kv1.3 channel in a rat experimental autoimmune encephalomyelitis model. The Journal of Biological Chemistry. 2012;**287**(35):29479-29494. DOI: 10.1074/jbc.M112.379594

[85] Pennington MW, Chang SC, Chauhan S, et al. Development of highly selective Kv1.3-blocking peptides based on the sea anemone peptide ShK. Marine Drugs. 2015;**13**(1):529-542. DOI: 10.3390/md13010529

[86] Cao Z, Yu Y, Wu Y, et al. The genome of *Mesobuthus martensii* reveals a unique adaptation model of arthropods. Nature Communications. 2013;**4**:2602. DOI: 10.1038/ncomms3602

[87] Varga Z, Gurrola-Briones G, Papp F, et al. Vm24, a natural immunosuppressive peptide, potently and selectively blocks Kv1.3 potassium channels of human T cells. Molecular Pharmacology. 2012;**82**(3):372-382. DOI: 10.1124/mol.112.078006

[88] Rodriguez de la Vega RC, Schwartz EF, Possani LD. Mining on scorpion venom biodiversity. Toxicon: Official Journal of the International Society on Toxinology. 2010;**56**(7):1155-1161. DOI: 10.1016/j.toxicon.2009.11.010

[89] Mouhat S, Andreotti N, Jouirou B, et al. Animal toxins acting

on voltage-gated potassium channels. Current Pharmaceutical Design. 2008;**14**(24):2503-2518

[90] Romi-Lebrun R, Lebrun B, Martin-Eauclaire MF, et al. Purification, characterization, and synthesis of three novel toxins from the Chinese scorpion *Buthus martensi*, which act on K^+ channels. Biochemistry. 1997;**36**(44):13473-13482. DOI: 10.1021/bi971044w

[91] Gao B, Peigneur S, Tytgat J, et al. A potent potassium channel blocker from *Mesobuthus eupeus* scorpion venom. Biochimie. 2010;**92**(12):1847-1853. DOI: 10.1016/j.biochi.2010.08.003

[92] Chen Z, Hu Y, Hu J, et al. Unusual binding mode of scorpion toxin BmKTX onto potassium channels relies on its distribution of acidic residues. Biochemical and Biophysical Research Communications. 2014;**447**(1):70-76. DOI: 10.1016/j.bbrc.2014.03.101

[93] Ye F, Hu Y, Yu W, et al. The scorpion toxin analogue BmKTX-D33H as a potential Kv1.3 channel-selective immunomodulator for autoimmune diseases. Toxins. 2016;**8**(4):115. DOI: 10.3390/toxins8040115

[94] Han S, Yi H, Yin SJ, et al. Structural basis of a potent peptide inhibitor designed for Kv1.3 channel, a therapeutic target of autoimmune disease. The Journal of Biological Chemistry. 2008;**283**(27):19058-19065. DOI: 10.1074/jbc.M802054200

[95] Shen B, Cao Z, Li W, et al. Treating autoimmune disorders with venom-derived peptides. Expert Opinion on Biological Therapy. 2017;**17**(9):1065-1075. DOI: 10.1080/14712598.2017.1346606

[96] Romi-Lebrun R, Martin-Eauclaire MF, Escoubas P, et al. Characterization of four toxins from *Buthus martensi* scorpion venom, which act on apamin-sensitive Ca^{2+}–activated K^+ channels. European Journal of Biochemistry. 1997;**245**(2):457-464. DOI: 10.1111/j.1432-1033.1997.00457.x

[97] Zhu L, Gao B, Luo L, et al. Two dyad-free shaker-type K^+ channel blockers from scorpion venom. Toxicon. 2012;**59**(3):402-407. DOI: 10.1016/j.toxicon.2011.11.016

[98] Wallace AN, Kansagra AP, McEachern J, et al. Evolution of endovascular stroke therapies and devices. Expert Review of Medical Devices. 2016;**13**(3):263-270. DOI: 10.1586/17434440.2016.1143772

[99] Man SM, Karki R, Kanneganti TD. Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. Immunological Reviews. 2017;**277**(1):61-75. DOI: 10.1111/imr.12534

[100] Kirk J, Plumb J, Mirakhur M, et al. Tight junctional abnormality in multiple sclerosis white matter affects all calibres of vessel and is associated with blood-brain barrier leakage and active demyelination. The Journal of Pathology. 2003;**201**(2):319-327. DOI: 10.1002/path.1434

[101] Ortiz GG, Pacheco-Moises FP, Macias-Islas MA, et al. Role of the blood-brain barrier in multiple sclerosis. Archives of Medical Research. 2014;**45**(8):687-697. DOI: 10.1016/j.arcmed.2014.11.013

[102] Peelen E, Damoiseaux J, Smolders J, et al. Th17 expansion in MS patients is counterbalanced by an expanded $CD39^+$ regulatory T cell population during remission but not during relapse. Journal of Neuroimmunology. 2011;**240-241**:97-103. DOI: 10.1016/j.jneuroim.2011.09.013

[103] Di Lucente J, Nguyen HM, Wulff H, et al. The voltage-gated potassium channel Kv1.3 is required for microglial pro-inflammatory activation in vivo. Glia. 2018;**66**(9):1881-1895. DOI: 10.1002/glia.23457

[104] Macrez R, Ali C, Toutirais O, et al. Stroke and the immune system: From pathophysiology to new therapeutic strategies. The Lancet Neurology. 2011;**10**(5):471-480. DOI: 10.1016/s1474-4422(11)70066-7

[105] Iadecola C, Anrather J. The immunology of stroke: From mechanisms to translation. Nature Medicine. 2011;**17**(7):796-808. DOI: 10.1038/nm.2399

[106] Chen YJ, Nguyen HM, Maezawa I, et al. Inhibition of the potassium channel Kv1.3 reduces infarction and inflammation in ischemic stroke. Annals of Clinical Translational Neurology. 2018;**5**(2):147-161. DOI: 10.1002/acn3.513

[107] Murray PJ, Allen JE, Biswas SK, et al. Macrophage activation and polarization: Nomenclature and experimental guidelines. Immunity. 2014;**41**(1):14-20. DOI: 10.1016/j.immuni.2014.06.008

[108] Saberbaghi T, Wong R, Rutka JT, et al. Role of Cl^- channels in primary brain tumour. Cell Calcium. 2019;**81**:1-11. DOI: 10.1016/j.ceca.2019.05.004

[109] Ullrich N, Sontheimer H. Biophysical and pharmacological characterization of chloride currents in human astrocytoma cells. The American Journal of Physiology. 1996;**270**(5 Pt 1):C1511-C1521. DOI: 10.1152/ajpcell.1996.270.5.C1511

[110] Olsen ML, Schade S, Lyons SA, et al. Expression of voltage-gated chloride channels in human glioma cells. The Journal of Neuroscience. 2003;**23**(13):5572-5582

[111] Mamelak AN, Jacoby DB. Targeted delivery of antitumoral therapy to glioma and other malignancies with synthetic chlorotoxin (TM-601). Expert Opinion on Drug Delivery. 2007;**4**(2):175-186. DOI: 10.1517/17425247.4.2.175

[112] Fan S, Sun Z, Jiang D, et al. BmKCT toxin inhibits glioma proliferation and tumor metastasis. Cancer Letters. 2010;**291**(2):158-166. DOI: 10.1016/j.canlet.2009.10.011

[113] Du J, Wang R, Yin L, et al. BmK CT enhances the sensitivity of temozolomide-induced apoptosis of malignant glioma U251 cells in vitro through blocking the AKT signaling pathway. Oncology Letters. 2018;**15**(2):1537-1544. DOI: 10.3892/ol.2017.7483

[114] Cheng Y, Zhao J, Qiao W, et al. Recent advances in diagnosis and treatment of gliomas using chlorotoxin-based bioconjugates. American Journal of Nuclear Medicine and Molecular Imaging. 2014;**4**(5):385-405

[115] King GF. Venoms as a platform for human drugs: Translating toxins into therapeutics. Expert Opinion on Biological Therapy. 2011;**11**(11):1469-1484. DOI: 10.1517/14712598.2011.621940

[116] Mamelak AN, Rosenfeld S, Bucholz R, et al. Phase I single-dose study of intracavitary-administered iodine-131-TM-601 in adults with recurrent high-grade glioma. Journal of Clinical Oncology. 2006;**24**(22):3644-3650. DOI: 10.1200/JCO.2005.05.4569

[117] Li S, Hou H, Mori T, et al. Swedish mutant APP-based BACE1 binding site peptide reduces APP beta-cleavage and cerebral Abeta levels in Alzheimer's mice. Scientific Reports. 2015;**5**:11322. DOI: 10.1038/srep11322

[118] Burgess A, Shah K, Hough O, et al. Focused ultrasound-mediated drug delivery through the blood-brain barrier. Expert Review of Neurotherapeutics. 2015;**15**(5):477-491. DOI: 10.1586/14737175.2015.1028369

[119] Kristensen M, Birch D, Morck Nielsen H. Applications and challenges for use of cell-penetrating peptides as delivery vectors for peptide and protein cargos. International Journal of Molecular Sciences. 2016;**17**(2). DOI: 10.3390/ijms17020185