Venom Peptides and their Mimetics as Potential Drugs

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Venomous creatures have a sophisticated mechanism for prey capture which includes a vast array of biologically-active compounds, such as enzymes, proteins, peptides and small molecular weight compounds. These substances target an immense number of receptors and membrane proteins with high affinity, selectivity and potency, and can serve as potential drugs or scaffolds for drug design.

Introduction

A large number of organisms produce and secrete venoms to defend themselves and to capture prey. Venom is a rich source of biochemically active enzymes, proteins, peptides and low molecular weight substances. Toxins isolated from the venom either inhibit or activate a vast number of targets such as ion channels, acetylcholine receptors, acetylcholinesterase, membranes, coagulant/anticoagulant pathways, and metalloproteases, with high selectivity and affinity. They can be roughly divided into non-peptide and peptide toxins. Non-peptide toxins have been isolated from algae, plants, dinoflagellate, fish and from higher organisms which accumulate alkaloids through their diet as exemplified in toxic frogs. Peptide toxins are generally synthesized in the venomous ducts of poisonous creatures. The majority of the data acquired to date has been from toxins isolated from the venoms of snakes, scorpions, spiders, marine snails (*Conus* genus) and sea anemones. Toxins isolated from venomous animals are usually small, ranging from 8-70 amino acids, with relatively small scaffold structures, which are highly compact and stabilized by either disulfide bonds or by hydrogen bonds made from post-transational-modified amino acids. In a number of toxins, the active residues responsible for the toxin activity have been identified, thus enabling the rational design of small molecular weight compounds or peptomimetics.

The pharmaceutical industry has recognized the enormous potential inherent in these venom peptides and has begun to exploit the selectivity and sensitivity fine tuned by evolution. This review will focus on peptides and toxin mimetics that are currently being evaluated as possible drugs for the treatment of pain, epilepsy, cardiovascular disorders, cancer and other neurological disorders (See Table), (for recent and comprehensive reviews please refer to references 1-10).
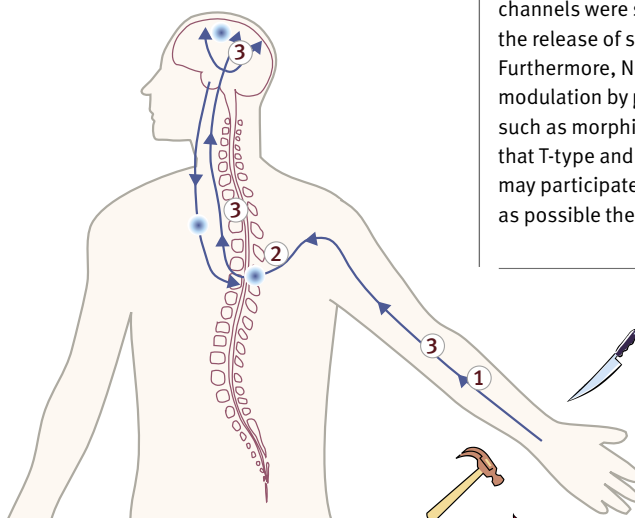
Pain

Recurrent pain was recently termed the "silent epidemic", since one out of six people in the western world suffers from pain, costing the American public alone approximately $100 billion each year in health care, compensation, and litigation.[11,12] Chronic pain is associated with conditions such as back injury, migraine headaches, arthritis, herpes zoster, diabetic neuropathy, temporomandibular joint syndrome, and cancer. Effective treatment options are limited to opioids (morphine and related drugs) and non-steroidal anti-inflammatories (NSAIDs), yet opioids have significant potential side effects, and NSAIDs are ineffective for moderate-to-severe pain. Pain can be generated by nociceptors stimulated by thermal, mechanical, chemical or inflammatory response, and a pivotal role for ion channels has been established[13] (please refer to the article "Contribution of Ion Channels in Pain Sensation" in this Modulator issue). Pain signals can be blocked at a number of sites along the pain pathway (See Figure). The large list of neurotransmitters and receptors identified along the pain pathway indicate that there may be many therapeutic possibilities for the pharmacological control of the transmission of nociceptive information to the brain.

Voltage-Gated Ca^{2+} Channels (VGCC)

Ca$_V$2.2 (α1B, N-type) was shown to control transmission at CNS and PNS synapses including in the transmission of pain signals at the spinal level. A PNS-specific Ca$_V$2.2 splice variant is highly expressed in the superficial layer of the dorsal horn, which is considered to be responsible for the nociceptive pathway of the spinal cord.[14] Ca$_V$2.2 was found to be up-regulated in the spinal cord during chronic pain states, along with the auxiliary α2δ-1 subunit.[15,16] Blockers of N-type channels were shown to block Ca^{2+} influx and thus the release of substance P in the spinal cord.[17] Furthermore, N-type channels are susceptible to modulation by μ-opioid peptide receptor agonists, such as morphine.[18] Recently it was proposed that T-type and possibly P/Q-Type Ca^{2+} channels may participate in pain pathways, and may serve as possible therapeutic targets.[14,19,20]

Pain pathways and pain signaling

1- Voltage-Gated Na$^+$ Channels
2- Voltage-Gated Ca^{2+} Channels
3- Neuronal Nicotinic Acetylcholine Receptors
   Noradrenaline Transporter
   Neurotensin Receptor Agonist
   NMDA Receptor Antagonist

Pain can be generated by nociceptors from a number of stimuli including, heat, cold, mechanosensation, inflammation, injury and pH. Venom toxins intersect pain signaling by inhibiting or stimulating channels and receptors along the pathway. The possible localization of the pain pathway targets are illustrated above.

A large array of VGCC peptide inhibitors were isolated from the venoms of cone snails, spiders and snakes. The most selective inhibitors of N-type Ca²⁺ channels known to date were isolated from *Conus geographus* (ω-**Conotoxin GVIA**), *Conus magus* (ω-**Conotoxin MVIIA**), and *Conus catus*, (ω-Conotoxin CVID). ω-Conotoxin MVIIA, a 25 amino acid peptide is a highly potent and selective blocker of N-type VGCC.[21] A synthetic ω-Conotoxin MVIIA analog, Prialt™ (previously called SNX-111 and Ziconotide) initially developed by Neurex Corporation and currently being commercialized by Elan Corporation for the treatment of severe chronic inflammatory and neuropathic pain associated with cancer and AIDS.[22,23] Although injected intrathecally, it was shown that Prialt™ is about 1000-fold more potent then morphine, lacking the tolerance or addiction usually associated with opiates.[24] However, Prialt™ administration results in severe side effects, including hypotension, sedation and confusion.[25] During December 2004, Elan Corporation was granted FDA approval for Prialt™ (ziconotide intrathecal infusion) for the management of severe chronic pain. ω-Conotoxin CVID analog, AM336, which is being developed by Amrad Corporation, has been in Phase II clinical trial since 2002 with cancer patients suffering severe chronic pain. AM336 was shown to inhibit a PNS-specific splice variant N-type VGCC associated with transmitter release from preganglionic nerve terminal and displays a wider therapeutic index than ω-Conotoxin MVIIA.[17,26] Recently, attempts were made to "convert" the toxin's pivotal binding residues into smaller molecular weight compounds, which might have superior pharmacological qualities in terms of formulation, production, metabolic stability and delivery.[27-31] One approach compared the

structure of ω-Conotoxin CVID against virtual screening libraries resulting in a cyclic peptide with D-amino acids having an IC$_{50}$ of ~20 µM and apparent selectivity between N- and P/Q- type VGCC.[27] Furthermore, using alkylphenyl ether based analogues which mimic three key amino acids of the toxins (Arg-10, Leu-11 and Tyr-13), leads to three compounds which have an apparent IC$_{50}$ of ~3 µM.[28] These and other experiments show that it is possible to design smaller entities which are active and selective.

Voltage-Gated Na⁺ Channels (VGSC)

VGSC modulators have been isolated from the venom of a variety of organisms, including spiders, sea anemones, scorpions, and cone snails.[1,8] There is a great need for specific venom peptides that can discriminate between the different VGSCs. A possibly abundant source for VGSC modulators was found in the venoms of cone snail venoms. Two main classes of conotoxins that affect VGSC are the µ- and δ-Conotoxins.[3,8] Recently the µ-Conotoxin SmIIIA toxin was isolated from the venom of *Conus stercusmuscarum*, which has been shown to block TTX-R currents in amphibian sympathetic and sensory neurons.[32,33] The authors speculate that the irreversible current block in the frog DRG was by inhibition of Na$_V$1.8 and Na$_V$1.9 channels, while the reversible inhibition of frog skeletal muscle is due to Na$_V$1.4 channel inhibition.[32] µ-Conotoxin SmIIIA or its mimetics may be an attractive toxin for pain treatment.

Neuronal Nicotinic Acetylcholine Receptors (nAChRs)

Nicotinic acetylcholine receptors (nAChRs) are a family of ligand-gated cation channels whose endogenous ligand is acetylcholine (ACh) and

which are activated by the alkaloid, nicotine. These pentameric conductance channels for Ca²⁺, K⁺, and Na⁺ are formed from a number of homologous subunits, and various combinations of these subunits result in channels which differ in their pharmacology and tissue distribution.[34] A class of conotoxins block nAChR and can differentiate between neuronal, skeletal muscle and sub-population isoforms of receptors, termed α- and αA- Conotoxins.[3,8,35] The latter toxins are termed *alpha* toxins as compared to a similarly acting α-Bungarotoxin, a blocker of muscle-type nAChR . Attempts to understand the mechanism of the selectivity have been reported.[36,37] The α-Conotoxins inhibit the signal transmission at the postsynaptic junction (neuronal or neuromuscular), by binding to the α-subunits of the nAChR, thus blocking the binding of acetylcholine and other agonists, which in turn inhibits the influx of Na⁺ required for action potential propagation.

α-Conopeptide Vc1.1 (other name ACV1), is a 16 amino acid peptide, containing two disulfide bonds. Its sequence was deduced from the nucleotide sequence of the conopeptide gene cDNA amplified from mRNA extracted from the venom duct of *Conus victoriae*. Vc1.1 was active as an antagonist of neuronal nAChRs in receptor binding and functional studies on bovine chromaffin cells. It also suppressed the vascular responses to C-fiber activation, and accelerated the functional recovery of injured peripheral nerves in rats. These peripheral unmyelinated sensory nerves are involved in pain transmission.[38] Recently, Metabolic Pharmaceuticals Ltd., who are developing ACV1 as an analgesic for the treatment of chronic neuropathic pain, has begun

rTamapin

A Novel Blocker of K$_{Ca}$2 (SK; small conductance Ca²⁺-activated K⁺) Channels

Tamapin (#RTT-400) is a 31 amino acid peptidyl toxin, isolated from the venom of the Indian red scorpion, *Mesobuthus tamulus*, and is classified as α-5.4 scorpion toxin family (P59869).[1,2] Native Tamapin blocks K$_{Ca}$2 channels in pyramidal neurons of the hippocampus as well as in cell lines expressing distinct K$_{Ca}$2 channel subunits. Tamapin displays a remarkable selectivity for K$_{Ca}$2.2 (SK2/KCNN2, IC$_{50}$=24pM) versus K$_{Ca}$2.1 (SK1/KCNN1, ~1750-fold) and K$_{Ca}$2.3 (SK3/KCNN3, ~70-fold) channels.[3] 500nM of rTamapin completely blocked K$_{Ca}$2.2 mediated currents in *Xenopus* oocytes.
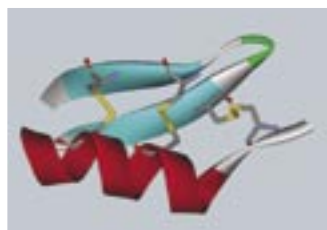
1. Doorty, K.B. *et al.* (2001) *Toxicon.* **39,** 137.
2. Rodriguez de la Vega, R.C. and Possani, L.D. (2004), *Toxicon,* **43,** 865.
3. Pedarzani, P. *et al.* (2002) *J. Biol. Chem.* **277,** 46101.

Model structure of Tamapin, based on its homology to scyllatoxin (1SCY). This class of peptides possesses the α-KTX-5 family scaffold, which serves as "poreplungers".
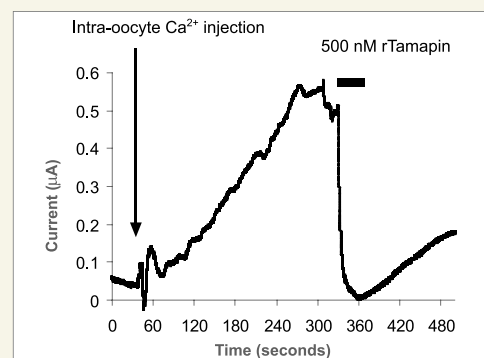
Activation of **rTamapin** (#RTT-400) sensitive outward current by intracellular Ca²⁺ injection into *Xenopus oocytes* expressing SK2 (K$_{Ca}$2.2) channels. The arrow and vertical bar represents time of intracellular injection and period of toxin perfusion, respectively.

preclinical and formal safety trials with this synthetic conopeptide. According to Metabolic Pharmaceuticals, preclinical trials show almost full relief of chronic pain with no apparent side effects and Phase I clinical trials are set to begin in early 2005.

Noradrenaline Transporter Inhibitors

Noradrenaline (or norepinephrine) (NE) participates in a number of biological pathways including the regulation of mood and sleep, expression of behavior, alertness and arousal. In episodes of pain, the elevation of NE levels in the spinal cord results in an inhibition of pain messages. The norepinephrine transporter (NET) returns noradrenaline to the synapses.

Among the A- super-family of conotoxins, the ρ- and χ-Conotoxins are known to modulate the α_1-adrenorecetor and neuronal noradrenaline transporter, exemplified by the two Conotoxins ρTIA and χMrIA isolated from *Conus tulipa* and *Conus marmoreus*, respectively.[3,8,39,40]

The synthetic χMrIA, termed Xen2174, acts by selectivity binding to NET and abolishing its ability to transport NE from the synapse back into the nerve ending. Xen2174, developed by Xenome Ltd, has recently entered Phase I clinical trials (intrathecal) for relieving nociceptive and neuropathic pain, while ρTIA is currently in preclinical trials.[3] Preclinical trial of Xen2174 in experimental animal pain models show no side effects and a high therapeutic index. Both ρTIA and χMrIA lack the common and often therapeutically limiting pharmacology of α_1-adrenoceptor antagonists (α_2-adrenoceptor and Na$^+$ channel inhibition) and NET inhibitors

(α_1-adrenoceptor and muscarinic ACh receptor antagonism), and thus may be useful clinically.[39,40]

Neurotensin Receptor Agonist

Contulakin-G is a novel 16-amino acid conopeptide originally isolated from the venom of the marine snail *Conus geographus*.[41] Cognetix Inc. is developing Contulakin-G (CGX-1160) for the short-term management of post-operative pain. CGX-1160 interacts with the neurotensin receptor 1 with 100-fold less potency (than neurotensin), but is 100-fold more potent as an analgesic, suggesting additional modes of action besides NT binding.[1] CGX-1160 has completed early Phase I safety studies in humans and has demonstrated efficacy in a broad range of preclinical models of acute and chronic pain.

NMDA Receptor Antagonist

Glutamate is the major excitatory neurotransmitter in the mammalian CNS. Upon release from presynaptic terminals, glutamate binds to postsynaptic ionotrophic receptors NMDA, kainite and AMPA. Glutamate acting on NMDA receptors is responsible for the initiation of CNS sensitization and hyperexcitability of spinal cord neurons upon nerve injury.[42,43] Non-specific NMDA antagonists relieve injury–induced pain, but have pronounced side effects.[43,44,45,50]

Conantokins were identified as a group of peptides that competitively inhibit glutamate activation, especially through NR2B or NR2B and NR2A subunits of NMDA receptors, and can discriminate between the different NMDA receptor types in the human brain.[7,8,43,46]

Conantokin-G, a 17 amino acid peptide isolated from *Conus geographus* selectively inhibits NR2B, while its related isoform, Conantokin-T, 21 amino acids long, isolated from *Conus tulipa*, inhibits both NR2B and NR2A receptors.[8,47] Both Con-G and Con-T lack disulfide bonds, and their structural stability is due to five post-translationally modified residues of the nonstandard amino acid γ-carboxyglutamate (Gla).[48,49] Cognetix Inc. is currently developing conantokin-G synthetic derivative (CGX-1007) as an anti-nociceptive drug and for control of seizures in intractable epilepsy, and is currently in Phase II clinical trials.[43]

Epilepsy

According to the Epilepsy Foundation of America, an estimated 1% of the total population suffers from epilepsy and seizures, afflicting more than 2.3 million Americans, with combined direct and indirect costs to the American economy of $12.5 billion. Total market volume of anti-epileptic drugs reaches $1.9 billion a year worldwide, with a 5% annual growth rate.

NMDA Receptor Antagonist

NMDA receptors have been shown to participate in a number of CNS malfunctions. CGX-1007 (see above) is currently in Phase II clinical trials as an anticonvulsant and for intractable epilepsy (when delivered directly into the central nervous system). The Phase I, randomized, double blind, placebo-controlled trial involved intravenous delivery of single, escalating doses of CGX-1007 in healthy, normal subjects to determine safety of the compound when administered to the systemic circulation. The results of the Phase I
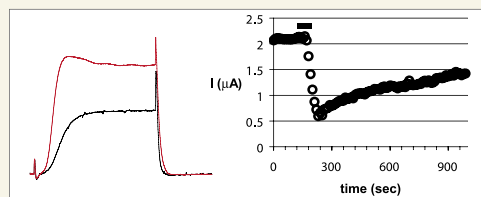
rStromatoxin-1

A Novel Blocker of K$_v$2 K$^+$ Channels

NEW

Stromatoxin-1 (ScTx-1) (#RTS-350) is a 34 amino acid peptide that belongs to the structural inhibitor cysteine knot spider peptide family. Electrophysiological recordings on COS cell expressing voltage-dependent K$^+$ channels show that this toxin acts as a gating modifier. Native ScTx-1 blocked K$_v$2.1, K$_v$2.2, K$_v$2.1/9.3 and K$_v$4.2, with IC$_{50}$ of 12.7nM, 21.4nM, 7.2nM and 1.2nM, respectively. No activity on K$_v$4.1 and K$_v$4.3 was observed.
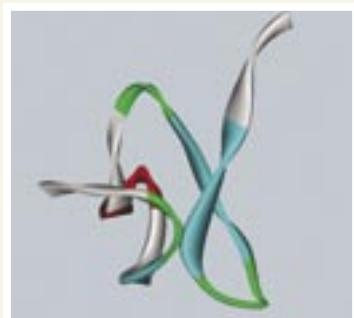
Model structure of ScTx-1, based on its homology to Hanatoxin1 (1DLH), SGTx (1LA4) and ω-Grammotoxin SIA (1KOZ). This class of peptides possesses the scaffold of structural inhibitor cysteine knot spider peptide family, which was shown to function as a gating modifier.

rStromatoxin-1 (#RTS-350) inhibition of K$_v$2.1 channels expressed in *Xenopus* oocytes. Left: current responses to 100 ms depolarization to 0 mV from holding potential of −100 mV, delivered every 10 seconds before (red) and during application of 670 nM rScTx-1. Right: time course of amplitude inhibition. The horizontal bars indicate the periods of rScTx-1 perfusion.

trial demonstrated that CGX-1007 was safe, with no clinically remarkable drug-related adverse experiences observed. Recently it was shown that although considered NR2B-specific, CGX-1007 is less specific or acts differently, than the investigational CI-1041 compound, in corneal kindled rats and in an NMDA receptor mediated excitatory postsynaptic currents model (N-EPSC).[44,45]

Stroke, Neuroprotection and Cardiovascular Disorders

Stroke and myocardial ischemia affect more than 65 million people in the United States and are the leading cause of death. Stroke is the leading cause of adult disability. Each year more than 700,000 Americans suffer a stroke, and one in five of them die. The total market volume of cardiovascular disorders drugs is estimated to be around $60 billion a year worldwide.[51]

Angiotensin Converting Enzyme (ACE)

ACE is an essential enzyme required for production of angiotensin, associated with hypertension due to vasoconstriction. Anti-hypertensive effect is achieved by inhibiting ACE. Capoten® (captopril) is a small molecular mimetic compound derived from a toxin found in the venom of the Brazilian arrowhead viper (*Bothrops*

jaracusa) developed by Bristol-Myers Squibb.[52]

Platelet Aggregation and Blood Clotting Inhibitors (inhibitors of platelet glycoprotein IIb/IIIa receptor)

Schering-Plough and Millennium Pharmaceuticals have been granted FDA approval for Integrilin® (Eptifibatide), a synthetic analog of barbourin, for the treatment of severe cardiovascular diseases, namely, anticoagulation in patients with acute coronary syndrome (ACS) and for patients without ACS undergoing percutaneous coronary intervention (angioplasty). Integrilin® is a heptapeptide derived from a protein found in the venom of the southeastern pygmy rattlesnake (*Sistrurus miliarius barbouri*).[53] Integrilin® acts as a parenteral platelet receptor glycoprotein IIb IIIa (GPIIb-IIIa) inhibitor and blocks platelet aggregation, a crucial event in thrombosis.

Aggrastat® (Tirofiban), developed by Merck, is also a GPIIb-IIIa inhibitor, however this drug was modeled on the structure of Echistatin, a derivative of the anticoagulant found in the venom of the African saw-scaled viper (*Echis carinatus*). Aggrastat® was the first GPIIb-IIIa inhibitor to be launched, but it is only approved for use with heparin and aspirin for the treatment of ACS.[54]

Viprinex™ (Ancrod), a compound isolated

from the venom of the Malaysian pit viper (*Agkistrodon rhodostoma*) is in late Phase III trials by Neurobiological Industries Inc. for use in the treatment of heparin-induced thrombocytopenia (deficit of platelets). Researchers found that blood failed to clot in animals bitten by these snakes. Viprinex™ removes fibrinogen from the blood, improving blood flow, a useful property that also has potential for the treatment of stroke.

Fibrin Thrombolytic Agent

Nuvelo Inc. is currently evaluating Alfimeprase, a synthetic version of fibrolase, a protein isolated from the venom of the southern copperhead viper (*Agkistrodon contortrix*), as an anticoagulant for the treatment of ischemic stroke and catheter occlusion. Alfimeprase, now in Phase II, was shown to directly degrade fibrin, producing a rapid dissolution of blood clots. Furthermore, Alfimeprase is currently in Phase II for catheter occlusion.

Thrombin Inhibitors

AstraZeneca is seeking FDA approval for Exanta™ (Ximelagatran) for treatment of patients with atrial fibrillation and patients at risk for blood clots. The orally active thrombin inhibitor was designed based on a cobra venom peptide. It is already on sale in Europe as a treatment to prevent blood clotting after orthopedic surgery.[55]

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rSlotoxin

A Novel Blocker of $K_{Ca}1.1$ (BK_{Ca}) K⁺ Channels

rSlotoxin (#RTS-410) is a 37 amino acid peptide toxin isolated from *Centruroides noxius*. Native Slotoxin blocks the Maxi K⁺ (BK_{Ca} or slo, KCNMA1, $K_{Ca}1.1$) channels. Slotoxin blocks differentially channels formed by the $\alpha1$ subunit alone and channels formed by the $\alpha1$ combined with auxiliary β subunits. In *Xenopus* oocytes expressing hSlo ($\alpha1$ alone), K_d was calculated to be 1.5 nM and the block is complete and reversible. With the additional co-expression of either the $\beta1$ or $\beta4$ subunits, the block becomes irreversible or causes the channel to be almost insensitive to the toxin, respectively.
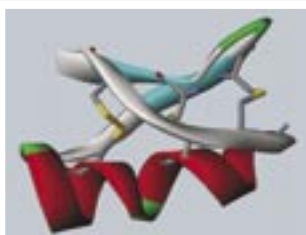
Model structure of Slotoxin, based on its Homology to Hongotoxin (1hlyA). This class of peptides possesses the α-KTX-1 family scaffold, which serves as "pore-plungers".
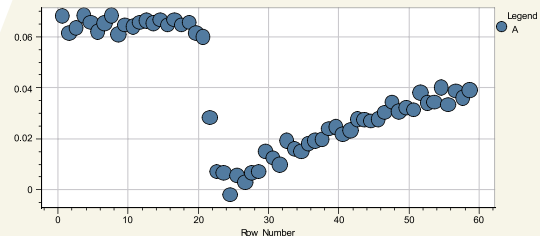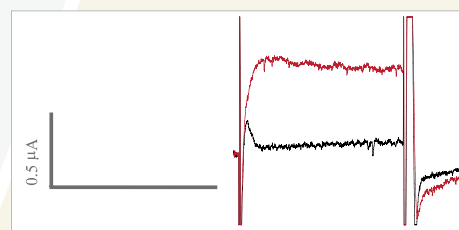
The effect of 100 nM **rSlotoxin** (#RTS-410) on heterologously expressed BK currents (mSlo, RNA injected to *Xenopus* oocytes). Lower: time course of current amplitude changes upon application of the toxin (bars represent the time of toxin perfusion Upper: An example of current response to 100 ms depolarization to +20 mV (from holding potential of −100 mV) before (red) and during (black) perfusion of 100 nM toxin.

NMDA Blockers

Delucemine (NPS 1506) is a compound being developed by NPS Pharmaceuticals as a means of protecting brain cells in ischaemia victims. Its structure was based on a spider venom toxin.[56,57] Phase I clinical trials with delucemine are currently underway in patients suffering from stroke and acute depression. NPS 1506 blocks NMDA receptors on neurons, thus preventing excessive Ca^{2+} influx during ischaemia.

Cancer

Current estimates by the American Cancer Society indicate that approximately 1.3 million individuals in the U.S. were diagnosed with cancer and there were about 500,000 cancer related deaths in 2002. Approximately $10 billion is spent on cancer drugs annually and cancer drug expenditures account for roughly 8% of total U.S. drug sales.

Cl⁻ Channels

Chlorotoxin is a 36-amino acid peptide that was originally isolated from the venom of the *Leiurus quinquestriatus* scorpion as a putative Cl⁻ channel inhibitor.[58] It was later found that Chlorotoxin could inhibit invasiveness of glioma cells *in vitro*. This inhibition was attributed to the ability of Chlorotoxin to block an unidentified Cl⁻ channel that was putatively involved in the process of regulatory volume decrease, a key step

Name	Peptide	Species	Target/ related protein	Disease	Clinical stage	Company
Synthetic/modified venom peptides						
Prialt™ (SNX-111, Ziconotide)	ω-Conotoxin MVIIA	*Conus magus*	Voltage-Gated Ca^{2+} Channels $Ca_v2.2$	Severe chronic inflammatory and neuropatic pain associated with cancer and AIDS	Granted FDA Approval (Dec. 2004)	Elan Corporation (www.elan.com)
AM336	ω-Conotoxin CVID	*Conus catus*	Voltage-Gated Ca^{2+} Channels $Ca_v2.2$	Severe chronic pain associated with cancer	Phase II	Amrad Corporation (www.amrad.com.au)
ACV1	α-Conotoxin Vc1.1	*Conus victoriae*	Neuronal Nicotinic Acetylcholine Receptors	Chronic neuropathic pain, and acceleration of recovery of injured neurons	Preclinical	Metabolic Pharmaceuticals (www.metabolic.com.au)
Xen2174	χ-Conotoxin χMrIA	*Conus marmoreus*	Norepinephrine transporter (NET)	Nociceptive and neuropathic pain	Phase I	Xenome Ltd (www.xenome.com)
	ρ-Conotoxin ρTIA	*Conus tulipa*	$α_1$-adrenoreceptor	Nociceptive and neuropathic pain	Preclinical	Xenome Ltd (www.xenome.com)
CGX-1160	Contulakin-G	*Conus geographus*	Neurotensin Receptor agonist	Short-term management of post-operative pain	Completed early Phase I	Cognetix Inc. (www.cognetix.com)
CGX-1007	Conantokin-G	*Conus tulipa*	NMDA receptors NR2B subtype	Nociceptive pain and control of seizures in intractable epilepsy	Phase II	Cognetix Inc. (www.cognetix.com)
TM-601	^{131}I-Chlorotoxin	*Leiurus quinquestriatus*	Cl⁻ channel	Brain tumors	Phase II	TransMolecular Inc. (www.transmolecular.com)
TM-701	I-Chlorotoxin	*Leiurus quinquestriatus*	Cl⁻ channel	Chronic monotherapy and pharmaceutical sensitizer co-administered drug cocktails for cancer	Preclinical	TransMolecular Inc. (www.transmolecular.com)
Alfimeprase	Fibrolase	Southern copperhead viper (*Agkistrodon contortrix*)	Fibrin	Thrombolytic agent and catheter occlusion	Phase II	Nuvelo Inc. (www.nuvelo.com)
	Contortrostatin	Southern copperhead viper (*Agkistrodon contortrix*)	Integrin	Breast cancer	Preclinical	Pivotal Biosciences/ University of Southern California (www.pivotalbiosciences.com)
Exenatide	Exendin-4	Gila monster (*Heloderma suspectum*)	Glucagon-like peptide-1	Type-2 diabetes and related metabolic disorders		Amylin Pharmaceuticals (www.amylin.com)
Peptomimetics or small molecular weight derivatives						
Capoten® (Captopril)		Brazilian arrowhead viper (*Bothrops jaracusa*)	Angiotensin Converting Enzyme (ACE)	Antihypertensive	Granted FDA approval	Bristol-Myers Squibb (www.bms.com)
Integrilin® (Eptifibatide)		Southeastern pygmy rattlesnake (*Sistrurus miliarius barbouri*)	Platelet glycoprotein IIb/ IIIa receptor Inhibitors	Acute coronary syndrome (ACS) and for patients without ACS undergoing percutaneous coronary intervention	Granted FDA approval	Schering-Plough Millennium Pharmaceuticals (COR Therapeutics) (www.schering-plough.com) (www.millennium.com)
Aggrastat® (Tirofiban)		African saw-scaled viper	Platelet glycoprotein IIb/ IIIa receptor Inhibitors	Acute coronary syndrome (ACS)	Approved for use with heparin and aspirin for the treatment of ACS	Merck (www.merck.com)
Viprinex™ (Ancrod)		Malaysian pit viper (*Agkistrodon rhodostoma*)		Heparin-induced thrombocytopenia	Phase III	Neurobiological Industries Inc. (www.ntii.com)
Exanta™ (Ximelagatran)		Cobra venom	Thrombin inhibitors	Atrial fibrillation and blood clotting after orthopedic surgery	Seeking FDA approval, sold in Europe	AstraZeneca (www.astrazeneca.com)
Delucemine (NPS 1506)		Spider venom toxin	NMDA blocker	Protection of brain cells from ischaemia	Phase I	NPS Pharmaceuticals (www.npsp.com)

in cell migration. Interestingly, Chlorotoxin was found to bind specifically to glioma cell lines and primary cultures, but not to normal brain cells.[59,60] Positive staining towards the labled-Chlorotoxin was found in other solid tumor cell lines, including non-small cell lung carcinoma, breast, prostate, melanoma, and colon cancers. Recent studies have shown that contrary to the original hypothesis, the specific target of Chlorotoxin on the surface of glioma cells might be matrix metalloproteinase-2 (MMP-2) protein and not a Cl^- channel.[61]   Recently TransMolecular Inc. has initiated Phase II clinical trials with its iodinated Chlorotoxin derivate TM-601(^{131}I-Chlorotoxin) for the treatment of brain tumors. TM-701, a derivative of TM-601, shares the same mechanism of action, but is used without a radioisotope. TM-701 is being developed as a chronic monotherapy and pharmaceutical sensitizer when administered with commonly used drug cocktails for treating cancer and is currently in preclinical trials. Other derivatives of TM-601 are being tested for use in *in vivo* imaging and diagnostic test kits.

Integrins

Integrins are a family of cell surface proteins, found on many cell types that mediate interactions between cells, and between cells and their surroundings. Specific integrin isoforms are upregulated during tumor growth.[71] Contortrostatin, a protein extracted from

the venom of the southern copperhead viper (*Agkistrodon contortrix*), binds to integrins on the surface of cancer cells and inhibits tumor growth and metastasis, while no cytotoxic effect on human breast cancer cells is observed.[62] PB2 (Contortrostatin), is cytostatic, 'freezing' tumor cells rather than killing them.[63] It is presently in preclinical research for the treatment of breast cancer by Pivotal Biosciences and the University of Southern California.

Diabetes

Glucagon-Like Peptide-1 (GLP-1)

Glucagon-Like Peptide-1 is an insulinotopic hormone secreted from endocrine cells of the small and large intestine in a nutrient-dependent manner. GLP-1 stimulates insulin secretion and modulates gastric emptying to slow the entry of ingested sugars into the bloodstream. The GLP-1 related peptide is a peptide initially derived from the salivary secretions of the Gila monster (*Heloderma suspectum*), a large venomous lizard. Amylin Pharmaceuticals is developing a synthetic version of Exenatide (synthetic exendin-4), a 39 amino acid peptide, currently in Pre-Phase III for use in treating type-2 diabetes and related metabolic disorders. Diabetic animal models have demonstrated that Exenatide is biologically active when administered via oral, sublingual, pulmonary, tracheal and nasal routes.

Furthermore, GLP-1 like peptides share structural homology to α-Latrotoxin, isolated from the venom of the black widow spider and might have potential in the treatment of Alzheimer's disease.[64,65]

Immunosuppressants and Autoimmune Disorders

The existence and participation of the voltage-dependent K^+ channel $K_V1.3$ and the Ca^{2+}-activated intermediate K^+ channel IKCa1 ($K_{Ca}3.1$) in T-lymphocyte activation is well established.[1,9] Furthermore, a marked elevation of $K_V1.3$ is reported in encephalitogenic T-cells, which mediate demyelination of axons in the brain and spinal cord, the hallmark of multiple sclerosis.[66] The use of specific blockers for K_V channels might have therapeutic potential for treatment of autoimmune disease, and as immunosuppressents for transplantations. In *in vitro* studies, the use of peptidyl toxins has indicated that blockage of $K_V1.3$ inhibits T-cell activation, suggesting that $K_V1.3$ may be a target for immunosuppression.[9] This concept was verified by *in vivo* experiments on peripheral T-cells of mini-swine using **Margatoxin** as specific $K_V1.3$ toxin.[67,68] Side effects of Margatoxin administration have been observed, mainly in the enteric nervous system which is expected for all non-specific $K_V1.3$ toxins.[1,9] Furthermore,

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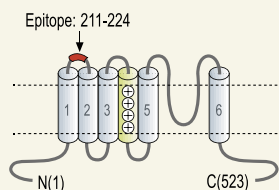# Anti-K$_V$1.3 Extracellular and K$_V$1.3 - Extracellular-FITC

$K_V1.3$ belongs to the *Shaker* family of voltage-dependent K^+ channels. The channel is widely expressed in the brain, lung and osteoclasts and in several cell populations of hematopoietic origin. It is in these cells (particularly T lymphocytes) that $K_V1.3$ function has centered a lot of attention. It was found that $K_V1.3$ is the main channel responsible for maintaining the resting potential in quiescent cells and regulating the Ca^{2+} signaling that is indispensable for normal T lymphocyte activation. Based on the

central role of $K_V1.3$ in regulating the initiation of an immune response, the channel has been recognized as a potential target for immunosuppressant drugs. The central role of $K_V1.3$ in immune system cells created a real need for a specific antibody that would be able to work in flow cytometry applications.

Alomone has now developed an anti-$K_V1.3$ antibody directed against a specific extracellular epitope of the

channel. The antibody can be obtained in a purified format (#APC-101) or labeled with fluorescein (#APC-101F). The antibodies have been tested successfully in flow cytometry, immunocytochemistry and western blot applications.

Epitope: 211-224

N(1)   C(523)

1   2

150
100
75

50

37

Western blotting of human Jurkat T cells:
1. **Anti-K$_V$1.3 (extracellular)** antibody (#APC-101) (1:500).
2. Anti-K$_V$1.3 (extracellular) antibody, preincubated with the control peptide antigen.

Anti-K$_V$1.3 *(extracellular)*-FITC
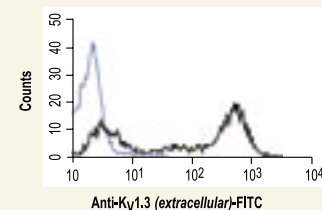
Flow cytometry analysis of Jurkat T-cells.
— Unstained cells
— Anti-K$_V$1.3 extracellular-FITC (#APC-101-F) (0.5 µl per 1 x 10^6 cells)

high serum concentrations of Margatoxin caused transient hyperactivity in pigs, indicating possible effects on $K_V1.1$ and $K_V1.2$ channels in the brain. Stichodactyla toxin (ShK), a toxin isolated form the venom of sea anemone *Stichdactyla helianthus*, has relatively similar affinities towards $K_V1.3$ and $K_V1.1$.[69,72] A ShK mutant, ShK-Dap[22] co-administered with TRAM-34, inhibitor of IKCa1, was tested in experimental autoimmune encephalomyelitis (EAE) animals (a model for multiple sclerosis), and was shown to effectively prevent lethal EAE.[9,66]

Conclusion

It is estimated that between 500 to 700 conus species exist, each possessing between 50-200 conopeptides in their venom. Thus, theoretically, over 50,000 pharmacologically active components can be found in the *conus* genus venoms, whereas only a small fraction has been studied to date.[8] Furthermore, the exact venom composition of other venomous creatures are planned to be sequenced in the Venom Genome project.[70] The precise protein-protein interactions between the venom peptides and the different channels might enlighten the possible selectivity issues that, in turn, might enable the design of more potent and selective toxins and of new small molecules with higher selectivities for new and safer drugs. Taken together, it seems that nature has evolved the venoms into a huge pharmacological library of active pharmaceuticals with high selectivities and affinities, which could be explored as therapeutics or serve as a template for drug design. The mechanism of action of each toxin family is different, thus each needs to be evaluated for its therapeutic potential. In conclusion, the large number of venom components may possibly serve as drug libraries, diagnostic tools, and for target specific research tools.

Acknowledgments

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