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Conotoxins: Potential Weapons from the Sea

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Abstract

Cone snails are predatory marine animals that kill their prey with powerful venom. Conotoxins are a pharmacologically and chemically diverse group of toxins found in the venom. A number of species of cone snails, such as *Conus geographus*, are deadly to humans. Conotoxins affect numerous neurotransmitter receptors and ion channels in the body. The receptors impacted include nicotinic, adrenergic, NMDA, and serotonergic. Ion channels altered include sodium, potassium and calcium. The most lethal effect of conotoxins to humans is muscle paralysis of the diaphragm causing respiratory arrest. Numerous conotoxins are being used as research tools or being explored as therapeutic drugs. Concerns in the homeland security field exist that certain conotoxins could be weaponized and used an aerosol. Conotoxins at risk of terrorist use include α-conotoxins, κ-conotoxins and δ-conotoxins. Most conotoxins are not a bioterrorism threat.

The Cone Snails

The cone snail is a marine predatory snail that uses powerful venom to kill its prey [1]. Conotoxins are a group of cysteine-rich peptide-based toxins in the venom of cone snails [2]. Most conotoxins contain multiple disulfide bridges. Secondary uses of conotoxins by the snails are protection against predators and competitors. Conotoxins are considered by the United States Centers for Disease Control and Prevention to be a potential agent in bioterrorism [3]. Most venomous animals (e.g. snakes and arthropods) only produce one or a few poisons. A single cone snail produces over 100 individual toxins [4]. Not all conotoxins are considered high risk for bioterrorism [5].

Cone snails belong to the phylum Mollusca, the class Gastropoda, the order Sorbeoconcha, and the family Conidae, and the genus *Conus* [6]. The shells of the cone snails are spiral and conic, hence their name. Cone snails are found in warm seas and oceans throughout the world but are mostly in the Indo-West Pacific region [7]. There are approximately 700 species of cone snails and all are carnivores. Most cone snails are nocturnal hunters. Different species specialize in eating fish, worms (e.g. polychaete), or other mollusks. The cone snails that eat fish are piscivores. Molluscivores eat other snails. Worm-eating cone snails are vermivores.

The siphon is the "nose" of the cone snail. The siphon is used for detecting prey and for respiration. The proboscis is the hunting tool used by the snail. The proboscis is a long tubular muscular elongation of the mouth. In the proboscis are harpoons containing the toxins. The synthesis of the conotoxins takes place in the epithelial cells of the venom duct and then secreted into the lumen of the venom duct. Attached to the venom duct is the venom bulb. The function of the venom bulb is to contract and push venom into the harpoon.

There are two types of piscivorous hunters: hook-and-line hunters and net hunters. The hook and line hunters use a proboscis with a harpoon containing toxins to paralyze their prey. The net hunters open their mouth to catch several fish at a time. Once inside the mouth a deadly harpoon kills the fish [8]. Each harpoon is discarded after use. The snails have approximately 20 harpoons at various stages of development.

Human Exposures

A sting by certain species of cone snails are poisonous to humans including *Conus geographus, Conus catus, Conus aulicus, Conus gloriamaris, Conus omaria, Conus magus, Conus striatus, Conus tulipa,* and *Conus textile* [9]. *Conus geographus* is the most lethal to humans [9]. Piscivores are more dangerous to humans than other cone snails [10,11]. From an evolutionary point of view the toxins must be stronger for piscivores than molluscivores or vermivores. Fish move much more rapidly than snails or worms. In order for a cone snail to be an effective predator of fish the toxins need to act instantly. Hunting a mollusk or a worm requires less speed. Fish are also zoologically more related to people than worms or mollusks.

Signs and symptoms of exposure include faintness, ptosis (drooping eyelids), poor coordination, absent gag reflex, areflexia, paresthesias (abnormal sensations such as burning or tingling), urinary retention, diplopia (double vision), blurred vision, speech difficulties, dysphagia (difficulty swallowing), weakness, nausea, generalized numbness, and respiratory arrest [9-13]. Autopsy findings may include blanching and swelling at the site of injection, petechial hemorrhages, cardiac dilation, and cerebral edema [12]. No specific antidotes are available. The heterogeneity in structure and the diverse pharmacology of the toxins are barriers to making an effective antidote. The treatment for a cone snail sting is respiratory support and intubation [13]. Vital signs, blood gases, and cardiac function need to be monitored. Death has been reported to occur within one to five hours [10,13]. The above toxidrome results from the interaction of a number of conotoxins rather than a single conotoxin.

Milder toxicity has been reported with molluscivorous and vermivorous cone snails. This is not to say that molluscivorous and vermivorous cone snails are harmless to humans. *Conus regius*, a molluscivorous snail, has been reported to cause paresthesias, numbness, and movement difficulty in the affected limb [14]. *Conus textile* is also a mollusk eater but considered hazardous to humans [9].

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Pharmacology of the Conotoxins

early laboratory studies found venom from *Conus geographus* to cause convulsions and respiratory suppression (without immediate concurrent cardiac arrest) in mice. In isolated muscles venom from *Conus geographus* produced muscle paralysis [15,16]. Conotoxins work on a variety of neurotransmitters in the body including glutamate, adrenergic, serotonin, and cholinergic and ion channels of sodium, potassium and calcium [17].

The conotoxins are classified by *Conoserver*, a database on conotoxins maintained by the University of Queensland, by gene superfamily, cysteine framework, or by pharmacological effects [18]. There are 18 gene superfamilies used by Conoserver [18] Classifying conotoxins by cysteines involves consideration of the number of cysteines in the peptide, their pattern, and their connectivity within the peptide. Pharmacological categories include alpha (α), gamma (γ), delta (δ), epsilon (ε), iota (ι), kappa (κ), mu (μ), rho (ρ), sigma (σ), chi (χ), and omega (ω) [18]. Refer to table 1 for the pharmacological characteristics of each class. Not included in the above classification system are the conantokins, which act on the N-methyl-D-aspartate receptors [19]. Conotoxins that modulate vasopressin/oxytocin receptors are also not included in the Conoserver classification system.

One of the key components to the venom of *Conus geographus* are the α-conotoxins [20]. The α-conotoxins are antagonists of nicotinic receptors. Nicotinic receptors serve a variety of functions in the body. Nicotinic receptors are needed for the contraction of skeletal muscle. Acetylcholine is released by a motor neuron. The acetylcholine then attaches to the nicotinic receptors on the muscle. This starts a physiological cascade causing the muscle to contract. Physically blocking the nicotinic receptor with a drug or toxin would stop the contraction and cause paralysis. The diaphragm is a muscle located below the lungs and divides the abdomen from the chest cavity. The diaphragm is the primary muscle that causes the lungs to inflate and deflate. Paralysis of the diaphragm results in the cessation of breathing. The diplopia reported from human exposures probably results from paralysis of the extraocular muscles.

Family	Physiological Effects
Alpha (α)	Blocks nicotinic receptors. Produces muscle paralysis
delta (δ)	Inhibits the fast inactivation of voltage gated sodium channels.
epsilon (ε)	Affects presynaptic calcium channels needed for action potential activity.
iota (ι)	Agonist at sodium gated channels with no delayed inactivation.
kappa (κ)	Antagonist of potassium gated channels. Interferes with repolarization.
mu (μ)	Antagonist of sodium gated channels.
rho (ρ)	Impacts alpha-adrenal receptors affecting blood pressure and smooth muscle.
sigma (σ)	Affects serotonin activity. Impacts mood, appetite and stress control
chi (χ)	Affects neuronal adrenergic transporter.
omega (ω)	Works on voltage gated calcium channels.
Conantokins	Antagonize glutamate, the main excitatory neurotransmitter in the brain, at N-methyl-D-aspartate receptors.
Conopressins	Modulate vasopressin/oxytocin receptors. Increases blood pressure.

Conotoxins are a pharmacological diverse group of toxins. Each group is selective for a specific neurotransmitter or cation channel.

Table 1: Pharmacological Classes of Conotoxins.

Nicotinic receptors are the main receptors at ganglia synapses. Nicotinic receptors are also found in the brain. The first isolated α-conotoxins were GI, GIA, and GII and found in *Conus geographus* [20]. These α-conotoxins do not affect the central nervous system. Other alpha-conotoxins were isolated different species including *Conus magus, Conus striatus, Conus consors, Conus achatinus,* and *Conus spurious* [21]. A number of α-conotoxins were found to have activity on the nicotinic receptors in the brain. The first centrally acting α-conotoxin was α-conotoxin CTx IMI isolated from *Conus imperialis*, a worm-eater [21].

Inhibiting the nicotinic receptor is not the only mechanism to cause muscle paralysis. Muscle paralyzing effects can be obtained by blocking the ion channels in the neurons supplying the muscles or by blocking the ion channels in the muscles. *Conus purpurascens*, is piscivore that causes both a flaccid paralysis and a "sudden tetanus on its prey" [22]. *Conus purpurascens* uses α-conotoxin PIVA and a μ-conotoxin PIIIA to paralyze its fish prey [22]. The α-conotoxin targets the nicotinic receptors in the muscles. The μ-conotoxin targets the sodium channels in the skeletal muscles [22]. This species also uses κ-conotoxin PVIIA and δ-conotoxin PVIA to cause the "sudden tetanus of prey" thus immobilizing the prey quickly [22]. All of the muscles of the victim fish are contracted at the same time. The fish experiences the pharmacological equivalent of an electrical shock. The κ-conotoxin PVIIA blocks potassium channels. Potassium is needed by the cells to reverse the action potential. Blocking the potassium channels prolongs the contraction. The δ-conotoxin PVIA delays sodium inactivation thereby prolonging the action potential. The δ-conotoxins from molluscivorous cone snails, with the exception, of Am2766, do not have active receptors in mammals [23]. The δ-conotoxins of piscivorous cone snails inhibit the inactivation of Nav channel in mammals [23]. Neurons, muscle cells, and cardiomyocytes require Nav channels for normal electrical functioning [24].

Scientific and Medical Applications of Conotoxins

Conotoxins are employed in both basic science investigations and for therapeutic explorations. Conotoxins are used as tools of research including determining how specific receptors and ion channels work. Conotoxins have potential roles in the direct treatment of disease. The ω-conotoxins are used in neuroscience research to study calcium channel subtypes [25]. A variety of conotoxins are used to understand specific sodium channels. A number of potential pharmaceuticals are being derived from conotoxins. Ziconitide is derived from *Conus magus* ω-conotoxin MVIIA. It has been approved by the United States Food and Drug Administration for treating intractable pain under the brand name Prialt®. K-conotoxin PVIIA may have cardioprotective effects [23]. Analogs of α-CTx MII, a centrally acting nicotinic blocker derived from *Conus geographus*, may have a role in treating Parkinson's disease [21]. Other centrally acting α-conotoxins in theory could be useful in treating Alzheimer's disease, nicotine addiction, and in pain management [21]. Prospective therapeutic or research uses of conantokins include pain, epilepsy, stroke, and Parkinson's disease. The chi family inhibits norepinephrine transport and thus is potential treatments for attention-deficit/hyperactivity disorder or depression [26]. Conotoxins that block Nav1.6 and Nav1.2 channels may mitigate the inflammatory process in multiple sclerosis [26]. Other conotoxins may have applications for studying schizophrenia [26]. There are an estimated 50,000 to 100,000 conotoxins and approximately 0.1% have been characterized pharmacologically [23].

Use as Terrorist Weapon

Conotoxins have potential as biological weapons [2,27]. The direct chemical synthesis would more likely be found in clandestine laboratory than the farming of cone snails. Collecting a large enough supply directly from cone snails to use in aerosol dispersal would be a cumbersome process. Most conotoxins are small peptides with 10 to 30 amino acids which make them relatively easy to manufacture using direct chemical synthesis [17]. Difficulties occur with the folding conotoxins producing discrepancies between in vitro and in vivo synthesis [28]. As discussed above, much research is being done with conotoxins. The supplies in laboratories could be diverted to terrorists. The United States Department of Health and Human Services requires registration, background checks, biosafety and security procedures for handling α-conotoxins at amounts exceeding 100 mg [29].

Potential methods of using of conotoxins in terrorism include contamination of food sources or aerial dispersal in a concentrated population area. The most likely method of dispersal would be as an aerosol [27]. Information on the inhalation effects of conotoxins is not available in the public domain. The onset of effects from inhaling conotoxins would probably be much faster than from cone snail stings assuming adequate absorption of the toxin in the lungs. Conotoxins are not volatile and need to be aerosolized. One barrier is creating the conotoxins as an aerosol is the developing the optimal particle size of 1 to 3 μm [30].

The mu family, the omega family, and NMDA antagonists are low risk for a bioterrorism incident [5]. Serotonin acting conotoxins are poor candidates for weaponization because obtaining lethal toxicity with serotonergic agents is difficult. The main predicted effect of adrenergic acting conotoxins based on the receptor activity would be a rapid increase in blood pressure. In theory, terrorists could also use certain conotoxins to disrupt agriculture by poisoning farm animals. However, conotoxins are not a "select agent" of the Animal and Plant Health Inspection Service of the United States Department of Agriculture and considered a low-risk for agricultural terrorism [3].

Botulinum Versus α-Conotoxins

From a pharmacological or toxicological point of view, the α-conotoxins are a high risk because of the muscle paralysis. The paralysis of the diaphragm results in respiratory arrest. The LD_{50} for α-conotoxins is 10 to 100 μg/kg in laboratory mice [31]. The LD_{50} for hydrogen cyanide is 1-3 mg/kg with oral ingestion [32]. Inhalation of certain α-conotoxins would be expected to produce a clinical presentation similar to the inhalation of botulism toxin. The clinical presentation of botulism poisoning from natural causes has many differences from a cone snail sting. Refer to table 2 for comparison of naturally occurring cone snail stings to natural botulism [33,34]. In addition to the clinical manifestations α-conotoxins have a different mechanism of toxicity from botulism toxin. Botulinum disables the terminals on the motor neuron [34]. The α-conotoxins work directly on the muscle. Regeneration of the nerve terminal by is required from botulism but not from α-conotoxins. That is why the recovery from botulism can take months.

The clinical onset of inhalational absorbed botulism or α-conotoxins would most likely be much faster than natural acquired intoxication. An early clinical differentiation of aerosol borne botulism from inhalational α-conotoxins may be difficult. Natural envenomations of cone snails involve a mixture of toxins whereas a terrorism situation may involve a single toxin. The cardiac manifestations associated with cone snail stings are probably not due to α-conotoxins. The numbness is not expected to occur with an exclusive exposure to α-conotoxins. Identification of the toxins in biological fluids would confirm the diagnosis. Most clinical laboratories lack the capabilities to analyze samples of conotoxins or botulinum toxin. Correct differential diagnosis between botulism and α-conotoxins is crucial. No specific antidote for α-conotoxins exists. An immunoglobulin based antitoxin is available to treat botulinum poisoning [2]. First responders probably would not be able to clinically distinguish inhaled α-conotoxins from inhaled botulinum. However, the prehospital treatment is the same i.e., maintaining an adequate airway and respiration. Again, the comparisons with botulism only apply to α-conotoxins with nicotinic activity in the muscle cells.

Naturally occurring Botulism	**Naturally occurring Cone Snail**
Frequently from ingesting improperly canned foods.	Handling or touching a cone snail and feeling a sting.
No numbness.	Numbness at site of sting.
Descending paralysis.	**Descending paralysis not described.**
Widespread muscle paralysis	Widespread muscle paralysis
No paresthesias or generalized numbness.	Paresthesias and generalized numbness.
Speech difficulties, urinary retention and double vision.	Speech difficulties, urinary retention and double vision
Death is often from respiratory arrest secondary to diaphragm paralysis.	**Death is from diaphragm paralysis or cardiovascular collapse.**
Onset is a matter of days.	Onset is a matter of hours.
Recovery takes months (without antitoxin). Recovery from botulinum F is a shorter duration.	**Recovery takes days.**

Table 2: Chart compares naturally occurring botulism (especially types A, B and E) to a cone snail envenomation. This comparison includes cone snails that produce α-conotoxins. Not all cases of botulism result from improperly canned food. The toxicity with cone snails will vary with species.

Other High-Risk Agents

The δ-conotoxins are also high risk because of the excitotoxicity and prolonged muscle contractions. κ-conotoxin PVIIA could cause cardiac toxicity by blocking the potassium channels in the heart.

Conclusions

The conotoxins are a vast range of poisonous substances produced by the predatory cone snails. Most conotoxins are not a bioterrorism risk. The α-conotoxins, κ-conotoxins and δ-conotoxins pose the greatest risk as terrorist threat. The most dangerous scenario is the clandestine manufacture of the toxins and delivering the toxins as an aerosol over a concentrated population area. Potential effects include muscle paralysis, muscle contractions, or other effects from altering ion channels in cardiac, nerve, or muscle cells. Numerous technical hurdles need to be overcome to weaponize the conotoxins.

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