Venom Insulin Derived from Cone Snails

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Cone snails possess a diverse array of novel peptide toxins, which selectively target ion channels and receptors in the nervous and cardiovascular systems. These numerous novel peptide toxins are a valuable resource for future marine drug development.

Keywords: cone snails; insulin; conoinsulin; diversity

1. Conoinsulin as a Weapon for Predation

Cone snails are commonly found in coastal reefs, rocks, and sandy beaches [42]. The shells of the cone snails are visually striking, featuring a diverse range of colors and bearing a resemblance to their non-toxic *Conus* counterparts. Consequently, they are occasionally inadvertently picked up by humans, resulting in potentially life-threatening incidents. There have been several documented incidents of accidental *Conus* collections leading to human fatalities [43]. Such poisonings are attributed to various toxins contained in the cone snail. This particular occurrence of poisoning is predominantly ascribed to various toxins manufactured within the cone snails. *C. geographus*, a proficient piscivorous predator, further exemplifies one of the most dangerous species to humans. Its venom can cause approximately 50% of poisoning incidents, as it encompasses 50% of the overall number of fatalities in all poisoning incidents [43]. Furthermore, *C. geographus* tends to result in higher mortality rates in children compared to adults [43]. Regardless of the victim's age, the poisoning incidents from larger snails are typically more prevalent than those from smaller snails. Additionally, humans have also suffered stings from other fish-feeding *Conus* species, leading to fatal injuries [43]. The venom produced by other *Conus* species that prey on gastropods also poses significant safety hazards to humans. However, most reported deaths have yet to be confirmed.

The unique predation methods of cone snails have attracted significant interest from many researchers [44,45]. Some cone snails employ a burying behavior, concealing their bodies within the sand while exposing their elongated proboscis.

This adaptive strategy enables them to simultaneously acquire oxygen and observe the activities of neighboring organisms. When detecting the proximity of potential prey, the cone snail deploys its elongated siphon beak in the direction of the prey [23]. Subsequently, the cone snails employ a specialized radular tooth with a hollow, harpoon-like structure, to immobilize the fish. This tooth not only anchors firmly onto its prey, but also injects a potent venom [23,44,45], acting much like a flexible “hypodermic syringe” connected to a sac filled with toxins [46]. By means of muscular contraction, the cone snail expels its venom into the prey’s body within a fraction of a second [47]. The venom discharged by the *Conus* species possesses potent toxic properties, capable of inducing severe poisoning or even lethality in the affected organism.

Fish rely on their inherent biological nervous system to regulate and coordinate their physical movements. When penetrated by the cone snail’s harpoon-like structure, the fish experiences a transient period of uncontrolled locomotion, lasting for a fraction of a second, followed by an immediate and complete paralysis [46]. The mixture of conotoxins contained in the venom swiftly targets the chemical receptors and ion channels responsible for modulating the fish's neural transmissions, causing the receptor to remain persistently open due to the influx of the toxin. The conotoxin, administered by the cone snail, induces muscular spasms in the fish. These toxins target the synaptic connections between the fish's nerves and muscles, impeding the muscle tissue's ability to receive command signals. Gradually, as the intensity of spasms diminishes, the fish typically succumbs to complete paralysis in the majority of observed instances [46,48]. Lastly, the cone snail retracts its proboscis and proceeds to maneuver the incapacitated prey towards its oral cavity, thereby concluding the entirety of the predation process.

Furthermore, an alternative predatory tactic employed by certain *Conus* species, referred to as *C. geographus*, involves the conspicuous expansion of their oral aperture, colloquially referred to as their “bloody mouths.” Once opened, these individuals commence the emission of venomous substances into the surrounding aqueous environment, effectively
paralyzing an entire assemblage of fish. These immobilized fish are subsequently engulfed by the cone snail in a single ingestion motion, facilitating the swift consumption of multiple prey items.

Within the venomous arsenal of *C. geographus*, a multitude of bioactive compounds are present, including insulin. This species uses specialized insulin in its venom to facilitate hunting [41]. Fast-acting neurotoxin and delivery systems are required for cone snails to use conoinsulin to capture prey [49]. After locking onto the prey, the insulin in the venom is rapidly propelled toward the bulbous base of the harpoon, where it is injected into the fish [50]. Relevant studies and data have shown a very fast delivery speed of cone neurotoxin [47]. Remarkably, *C. geographus* produces two distinct forms of insulin. One resembles molluscan insulin and is produced in the nerve ring and the esophagus, which play a role in the regulation of hemolymph glucose levels, memory and learning.

### 2. Diversity Analysis of Conoinsulin

Insulin-like peptides, identified in cone snail venom, exhibit species-specific variations regarding expression. Certain species exclusively express a single type of conoinsulin, whereas others possess multiple conoinsulin variants. The precursors of these conoinsulins typically feature a conserved N-terminal signal sequence in their amino acid sequences. Among these sequences, some contain a pro-peptide region, while others did not; however, all these sequences exhibit at least one amino acid difference in the mature region [54]. Cone snails deploy venoms containing conoinsulin, which acts within seconds to immobilize nearby fish, facilitating easier capture and consumption [41,55]. This rapid action of conoinsulin, in stark contrast to human insulin, has intrigued scientists. In the ConoServer and UniProt databases, 38 different insulin sequences and one insulin-like peptide have been reported in the venom of 18 types of *Conus*, and alignment analysis was performed using MEGA 7.0.14. Con-Ins G1, Con-Ins G1b, Con-Ins G1c, Con-Ins G2, Con-Ins G2b, Con-Ins G3, and Con-Ins G3b were all sourced from *C. geographus*; Con-Ins T1A, Con-Ins T1B, Con-Ins T2, Con-Ins T3, and Con-Ins T4 were all discovered in *C. tulipa*; Con-Ins K1 and Con-Ins K2 were derived from *C. kinoshitai*; Con-Ins Q1 and Con-Ins Q1b were both sourced from *C. quercinus*; Con-Ins F1, Con-Ins F2, Con-Ins F2b and Con-Ins F2c were derived from the species *C. floridulus*; Con-Ins Me1 was discovered in *C. memiae*; Con-Ins Im1 and Con-Ins Im2 were found in *C. imperialis*; Con-Ins Pa1, Con-Ins Ti1, Con-Ins Pu1, Con-Ins Vir and Con-Ins Bn1 were discovered in *C. planorbis*, *C. tribblei*, *C. pulicarius*, *C. virgo* and *C. bandanus*, respectively; Con-Ins V1 and Con-Ins V2 were found in *C. varius*; Con-Ins Ts1 and Con-Ins Ts2 were found in *C. tessulatus*; Con-Ins Eu1 and Con-Ins Eu2 were discovered in *C. eburneus*; Con-Ins Mr1 and Con-Ins Mr2 were discovered in *C. marmoreus*; Con-Ins Tx1 and Con-Ins Tx2 were discovered in *C. textile* and ILP was discovered in *C. victoriae*. The precursors of human insulin, zebrafish insulin, and conoinsulin are all composed of signal peptides and mature peptides [21,56].

Insulin, a natural hormone, has been proven to manifest an extensive assortment of aggregates with diverse structures and morphologies [51-59]. For instance, the mature region in insulin represents a functional domain encompassing both A, B and C chains, typically characterized by variability and interconnected through disulfide bonds, thereby facilitating the exertion of its pharmacological activity [56,60,61,62]. Like human insulin, the mature peptide of conoinsulin contains chains, albeit with notable differences. In insulin sequences, cysteine residues, which are largely conserved, predominantly cluster in the A and B chains. Typically, the A chain of insulin follows a CC-C-C cysteine pattern, while the B chain has a C-C pattern, leading to the formation of three disulfide bonds. In contrast, some conoinsulin A chains exhibit a C-CC-C-C pattern, while the B chain has a C-C-C pattern, resulting in four disulfide bonds. In addition, the human insulin B-chain contains a C-terminal segment, which plays an important role in the assembly of insulin dimers or hexamers [63,64,65,66]. Interestingly, some conoinsulins such as Con-Ins G1, despite the noticeably short or partially absent C-terminus of the insulin B chain, still retain an affinity towards the human insulin receptor [52,67,68]. These findings imply that the C-terminal of the B-chain does not serve as a pivotal determinant influencing the binding affinity between insulin and the insulin receptor [67]. Moreover, the conoinsulin does not undergo dimerization or hexamerization, thereby allowing for rapid reaction kinetics surpassing those of current insulin medications. Cone snails produce conoinsulin not only for regulating blood sugar like most organisms, but also for predation purposes.

The three-dimensional (3D) structure of Con-Ins G1 from *C. geographus* was solved using X-ray crystallography [71]. Con-Ins-G1 not only exhibits a high affinity for the human insulin receptor but also shares a striking similarity with the zebrafish insulin [41]. Biologically, Con-Ins G1 has the highest similarity to fish insulin [71], particularly in the A-chain, while the B-chain similarity is not as pronounced [41]. In addition, owing to its compact structure, Con-Ins G1 acts swiftly [72], which aligns well with the rapid predation strategy of cone snails. Using Con-Ins G1 (PDB 5JYQ) as a template, homologous modeling methods generated nine different conoinsulin variants corresponding to the dietary preferences of various cone snails, including G1b, G3, T1b, K1, F1, F2, Im1, Tx1, and Mr1. Meanwhile, 3D modeling of zebrafish insulin using human insulin (PDB 3I40) as a template revealed highly homologous structures [63,73,74,75,76].
Through structural comparison, analysis reveals that all insulin molecules contain three α-helices, with two located in the A-chain and one in the B-chain. Additionally, they feature a hydrophobic core composed of non-polar residues, vital for their proper folding and structural integrity [77, 78]. The conoinsulins from fish-hunting cone snails display a high degree of structural similarity with human insulin and zebrafish insulin. This resemblance likely correlates with their dietary characteristics, aiming to act on fish IRs to lower blood sugar levels for effective predation.

3. Evolutionary Relationship of Conoinsulin

Conoinsulin expression is ubiquitously observed in mollusk-hunting, worm-hunting, and fish-hunting Conus individuals. However, unlike mollusk-hunting Conus and worm-hunting Conus, only those fish-hunting Conus that use a net-hunting strategy express venom fish-like insulin [53]. Hence, among some fish-hunting Conus species, unique insulin variants serve as the principal components of their venomous arsenal, conferring a critical advantage in prey capture.

Con-Ins Ti1, found in the worm-hunter C. tribblei, represents the most primitive venom insulins of cone snails. Subsequently, the phylogenetic tree diverged to include venom insulins produced by cone snails which specialize in feeding on fish and those that prey on snails. Among these fish-hunting Conus, the earliest appearing insulins are Con-Ins G2 and Con-Ins G2b originating from C. geographus. The venom insulins produced by cone snails with a fish-based diet, particularly the Con-Ins G1 originating from C. geographus, have attracted significant attention in recent research. It is noteworthy that the abundance of venom insulins from fish-hunting cone snails surpasses others, constituting approximately half of the phylogenetic tree encompassing Conus venom insulin. Following this, venom insulins produced by cone snails specializing in worm consumption display a moderate presence, whereas the quantity of venom insulins from mollusk-hunting cone snails remains relatively minimal.

According to the literature research, it has been found that all mollusk-hunting cone snails, most but not all worm-hunters and only a small subset of fish-hunters express conoinsulins [53]. Differing prey capture strategies may explain this difference. Some fish-hunting cone snails appear to release conoinsulins into the water to make an entire school of small fish hypoglycemic, thereby enhancing the cone snail's ability to engulf multiple fish. In contrast, some fish-hunting species with no conoinsulins mainly capture fish by producing complex conotoxins that by cause hyper-excitability of the nervous system and rapid onset of tetanic paralysis. There may be no role for a conoinsulin in this prey-capture strategy. Additionally, several worm-hunting species exhibit low or no levels of conoinsulin expression, indicating that conoinsulin may no longer be important in these species [51].

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