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Histological study of the venom production organ in *Conus coronatus* and *Conus frigidus*

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Abstract
We were studied the venom apparatus of two marine cone snails, specimens of *Conus coronatus* and *Conus frigidus* were collected from the Coast of Qeshm Island, Persian Gulf. After dissection, the venom apparatus were fixed in Bouin's fixative, for the histological purposes. The venom bulb is a muscular organ which, located in the middle part of a channel with epithelial cells that secreted some material. Venom duct walls consists the outer layer of connective tissue with muscle, an inner layer of columnar epithelial cells with basal nucleus and the inner lumens with the departed nucleus. The distal portion of the venom duct displayed fewer granules than the proximal in both species. Although Holocrine secretion was find in the whole venom apparatus, but venom bulb had a weak secretion role. The venom duct near the pharynx probably had a more mature granule than the other part.

Keywords: Conus, Holocrine, Venom apparatus, Granules

1. Introduction
Predatory marine snails of the genus Conus is important because of their highly evolved hunting strategies employ peptide toxins that paralyze prey. Cones utilize a long, flexible, hydrostatically-supported appendage, the proboscis for prey [1-3]. The proboscis has a conduit to deliver immobilizing venom, whose composition can vary both interand and intraspecifically [4-6]. To envenomate prey, cone snails inject a harpoon-like a radular tooth into their prey, allowing toxins to be delivered through the hollow central canal of the tooth [7]. It is not clear how the proboscis generates the pressure necessary to propel the tooth into prey. One possibility is that the muscles of the proboscis contract the fluid-filled lumen to generate pressure [7, 9]. Approximately 700 *Conus* species exist in the world, [10] all of them are venomous with different toxicity [11, 14]. *Conus* toxins are typically 10–30 amino acids in length and belong to one of several families defined by highly conserved cysteine frameworks and internal disulfide linkages [4, 11, 14]. The venom of each species contains up to 200 pharmacologically active components that mainly target different voltage- and ligand-gated ion channels [12, 15]. Production and delivery of Conus venom involve three general steps: (1) synthesis, processing, and packaging of peptide toxins; (2) generation and storage of radular teeth and transfer of a tooth to the tip of the proboscis; and (3) the final insertion of the tooth and ejection of venom. These processes are carried out within an anatomically complex venom apparatus in conjunction with the anterior regions of the digestive tract [16]. Production of venom takes place in a long, convoluted venom duct. The proximal end of the duct is equipped with a muscular bulb. Distally the duct enters the pharynx just anterior to its border with the esophagus. Proximal and distal portions of the duct differ in gross structure and venom content [16, 18]. The muscular bulb is generally thought to take little secretion of venom. The bulb has often been hypothesized to provide the force for final venom ejection out of the tooth [11, 13, 19]. It is more likely that the muscular proboscis provides the necessary positive pressure to mediate venom ejection [2, 3, 20]. With each Conus species producing a distinctive repertoire of 100-200 venom peptides [21, 22]. More than 100 conotoxins purified from venoms have been classified into pharmacological families according to their molecular targets [21], More than 300 venomous species are known of which forty are dangerous and believed to cause poisoning in human [23, 24].

Toxins included in these venoms are being studied for understand the mechanisms underlying the evolution of species interactions, ecological diversification and the functioning of the nervous system, and also for potential therapeutic applications. Present study is aimed to know the biology of venom production in *Conus coronatus* and *Conus frigidus*. This report describes some features of the venom apparatus as revealed by histological study.

2. Materials and methods

Specimens of *C. coronatus* and *C. frigidus* were obtained from Zeyton Park, Qeshm Island, and Persian Gulf. N 26 55' 631", E 56 15' 209". The length of specimens: *C. coronatus* and *C. frigidus* ranged from 2.3-4.6 cm and 3.7-6.8 cm, respectively. The shells were cracked with vise-grips and removed. The complete venom duct was dissected under seawater. Tissue samples were fixed in Bouin's fixative for 48 hours and then dehydrated in a graded ethanol series. Tissues were then cut into three pieces (bulb, proximal, distal) and embedded in Paraffin, then were cut with a Leica RM2245 microtome, stained with Haematoxylin-Eosin and examined with an Olympus CH2 microscope. Using at least photos magnified (40X), the number of granules was estimated. These images were analyzed using Image Tool (2, 0) software. The data were analyzed using SPSS software version 11.5. All the data were expressed as mean ± standard error. The results were significant at $P<0.05$. Differences between two portions (proximal and distal) in each species was done by student t-test.

3. Results

Present study was conducted on three portions (bulb, proximal, distal) of *C. coronatus* and *C. frigidus* venom apparatus. Venom duct was long, twisty and yellow that was about 12 cm in *C. coronatus* with an average shell, 2.8 cm and 15 cm in *C. frigidus* with an average shell, 5.4 cm (Fig. 1A-B and Fig. 2A-B). The structure of venom apparatus was similar in both species, the venom bulb was located in the first part of venom apparatus, which, composed of circular and longitudinal muscles and in their middle part a channel with epithelial cells that secreted some material. Mostly venom bulb space is composed of longitudinal and the circular muscles and the inner layer of cells make up only a small part of it (Fig. 1 and Fig. 2).

Near its junction with the muscular bulb, was the proximal venom duct. Light microscopy reveals three distinct zones in this region: the outer layer of connective tissue with muscle, an inner layer of columnar epithelial cells with basal nucleus and the inner lumens which filled with the departed nucleus of holocrine secretion in all parts of venom apparatus.

The distal venom duct is about one-quarter of the distance to the pharynx, Extracellular granules within the distal duct lumen in this region are substantially more abundant than in the proximal region. (Fig. 3 and Fig. 4).

The number and granules was calculated using Image Tool (2, 0) software. The proximal portion of the venom duct showed lower number granules than the distal portion in both species

Table 1. Mean number of the granules in proximal and distal portion

	C. coronatus	*C. frigidus*
Distal Portion (0.05 mm^2)	82 ± 8^{A}	$102 \pm 11^{*}$
Proximal Portion (0.05 mm^2)	135 ± 11^{B}	$162 \pm 16^{**}$

Values are presented as the Mean±S.E for each portion. Different letters of each column indicate significant difference ($P< 0.05$).

4. Discussion

The cone snail (genus Conus), a marine gastropod lives mainly in the tropical habitat of shallow waters near coral reefs. All species are venomous with different toxicity [11, 13, 25, 26]. As described in this paper, the general anatomy of the venom apparatus of *C. coronatus* and *C. frigidus* is similar to that in other Conus species. Few venom granules or obvious precursor materials exist in the lumen of the distal duct of these two species, and this suggests that venom granules are manufactured elsewhere, presumably in more anterior duct regions. Numerous venom granules characterize the entire proximal venom duct, and they are found both within epithelial cells and in the lumen. These features strongly suggest that venom granules originate in the proximal venom duct [1, 16, 17]. The epithelial cells of this region probably express the genes encoding specific peptides, and granules appear to be assembled in the same cells [5, 6, 16]. Previous studies have indicated that material extracted from the proximal portion of the *Conus* venom duct has different peptides, venom granules, and pharmacological properties than that extracted from the distal duct [18, 19, 22]. *Conus californicus* has the distal venom duct is composed of a well-organized epithelium that is highly specialized with a lumen contains a great deal of cellular debris and few venom granules and the proximal venom duct is composed of a poorly organized epithelium [16]. In the present study both cellular debris and venom granules was observed in the lumen [16], as we observed in this study.

Within a cone snail, a muscular bulb connects to a long tubular venom duct opposite from the duct's site of insertion into the pharynx and plays a central role in pressurization of the proximal proboscis lumen and delivery of venom into the pharynx [3, 9, 16, 19]. The biosynthesis of certain conotoxins are probably associated with the specific type of epithelial cells found in different sections of the venom duct. The biological observations of Endean and co-workers and our own observations indicate greater toxicity of venom from the proximal portion compared to the distal portion [19]. This could only be true if there is a difference of toxin levels in the two regions. Another possible level of control and source of diversity is the post-translational modification of peptides in the Conus venom. The spectral counts of both unmodified and posttranslationally modified conotoxins show the quantitative variation in extent of processing. The varying levels of modification for each conotoxin could represent under processed venom component or deliberately produced variants to increase conotoxin diversity [18].

5. Conclusions

The combinatorial strategy of Conus venom presents a perfect scheme for the successful ecological adaptation and envenomation of prey and competitors by cone snails. The snail's venom bulb is believed to be involved in the transport and delivery of the potent venom components. It may be able to control the volume of venom injected and determine whether the components injected come only from the distal section or includes components from the central and proximal sections. And difference level of toxicity between proximal and distal portion of the venom duct maybe is correlated to the post-translational modification of peptides in the Conus venom

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