Histological aspects of the venom glands in *Vipera ammodytes montadoni*

Ivona POPOVICI, Carmen SOLCAN
University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad", Iași
ivona.laiu@yahoo.com

Abstract
Venom is produced by certain snake families and is used by these to capture the prey and to defend themselves when they feel threatened. The venom contains a variety of biomolecules including hyaluronidases, metalloproteinases, myotoxins, phospholipases and neurotoxins. These enzymes can cause on envenomed persons a large range of symptoms from local pain, swelling and necrosis to systemic disorders like tachycardia, coagulopathy, neurotoxicity, respiratory paralysis and even death if an appropriate treatment is not administrated. In this study we describe the histological structure of the venomous glands in snakes of Vipera ammodytes montadoni. Venom is produced by modified oral glands on each side from which one is the main glands and one is the accessory. All the glands presented a simple secretory epithelium with several types of cells. The main glands are modified oral glands presenting large secretory units with cell presenting secretory granules accumulated in cytoplasm. In the connective tissue surrounding the acini many blood vessels and also melanocytes were observed. The accessory gland presented two regions made up of secretory acini with large lumens filled with a PAS-positive secretion. The acini of the accessory gland presented several types of cells. Skeletal muscle fibers from the compressor muscle were observed in the region of the main and accessory glands. The secretion from these glands was collected into primary and secondary ducts lined by a simple columnar epithelium.
Keywords: *oral glands, snake, venom, Viperidae*

Introduction

In certain snake families modified salivary glands produce a mixture of proteins, peptides, biogenic amines and carbohydrates, the venom, that is used to capture the prey and to defend themselves when they feel threatened. The biomolecules in venom have enzymatic and nonenzymatic activities and a synergic activity in potentiating their toxicities in the envenomated organisms (Xiong and Huang, 2018). From the biological active molecules of the venom more than 30% are represented by metalloproteinases which have hematologic effects and interfere with the blood coagulation cascade causing a variety of symptoms such as haemorrhage, hypotension and hypovolemia (Takeda et al., 2012). Tissue destruction is caused by enzymes such as phospholipases, hyaluronidases, myotoxins and neurotoxins. Phospholipases A2 are a family of lipolytic enzymes that act upon the phospholipids in the cellular membrane in the presence of Ca ions (Kang et al., 2011). Hyaluronidases are venom enzymes which degrade hyaluronic acid, a glycosaminoglycan in extracellular matrix of tissues (Waheed et al., 2017). The venom myotoxins act upon the muscle fibers and may lead to paralysis, which can also be caused by neurotoxins that interfere with the neuromuscular transmission (Gasanov et al., 2014).

Despite their toxic effects on bitten victims, the studies revealed that the venom components can be used for bacterial infectious treatment, cancer (Arruda Macedo et al., 2015; Calderon et al., 2014) and inflammation treatment (Silva et al, 2019), in hypertension, in antithrombotic therapy (Bledzka et al., 2013), for haemostasis (Wei et al, 2010), for pain management (Pu et al., 1995).

The role of the L-amino acid oxidase in the platelet aggregation is controversial (Kang et al, 2011), but the studies revealed that this flavoenzyme oxidase that is made up of three domains

has antimicrobial, antitumor and antiprotozoal effects (Costa et al., 2014; Izidoro et al., 2014). The antibacterial effects of venom are due to several components such as the LAAO and omwaprin and can be used as an alternative to the antibiotic treatments (Nair et al., 2007; Gomes et al., 2005).

In snakebite envenoming the treatment depends on the administration of antivenoms, but because there are problems regarding the high costs of manufacturing it and also the adverse reactions that appear in some cases new approaches are being pursued (Laustsen et al., 2016).

In south-eastern Europe, the nose-horned viper (*Vipera ammodytes*), is considered to be the most dangerous snake (Chippaux J., 2012).

The venom is produced by the venom glands. Both the venom and the venom gland are part of the venom apparatus along with the compressor muscles, the venom teeth and the behavior and has the role to produce and deliver the venom (Jackson K., 2003). The venom glands are situated along the upper jaws and are composed of an anterior accessory gland and a posterior main gland. There is a primary duct that make the connection between the two. A secondary duct leads the venom from the accessory gland to the canal situated in the fang.

Materials and methods

The unextracted venomous glands along with other components of the venom apparatus were studied in a female and male snake from *Vipera ammodytes montadoni* subspecies which were raised in captivity for venom extraction. The samples were fixed in Bouin solution, decalcified with EDTA for 3 weeks, dehydrated using several baths of ethanol, cleared in xylen, and embedded in paraffin. Sections of 5µm were cut using a Slee microtome, and stained with hematoxylin and eosin and periodic acid Schiff.

Results and discussions

The unextracted main gland, the most voluminous part of the venom gland was covered by striated muscle fibers forming the compressor muscle (*Figure 1*). When this muscle contracts, the pressured main gland releases the stored venom (Jackson, K. 2007). The main venom gland located posteriorly was made up of branched tubular cisternae surrounded by thin strands of connective tissue with melanocytes (*Figure 2*). The venom appeared as a PAS positive secretion in the lumen of the unextracted secretory units (*Figure 2*). The secretory epithelium was represented by cuboidal cells.

The appearance of the secretory cells in the main gland differ depending on the secretion status of these cells. When the cells are involved in synthesizing the components of the venom, the cells become tall columnar and decrease in high in the periods when the gland stores the venom (Mackessy and Baxter, 2006). During the venom synthesis period cells having the same appearance as the parietal cells found in the gastric pits of the mammalian stomach can be observed. The role of the cells is to low the pH. In the stomach, a low pH has the role to activate the digestive enzymes but in the venom gland the acidification of the secretory product inactivates the enzymes contained by the venom, making it harmless for the glandular structures (Mackessy and Baxter, 2006).

From the secretory units the venom went to the central lumen of the main gland where it was stored. Under the contraction of the compressor muscle the venom is sent from here into a primary duct which is associated with an accessory gland (Viana et al., 2017).

Histologically, the accessory gland (*Figure 3*) presented two different parts: a posterior part in which the epithelium was cuboidal to columnar and an anterior region with mucous epithelium. In the posterior region epithelium showed a complex structure, several types of cells being observed in here. There were noticed tall columnar cells with acidophilic cytoplasm and

flattened nuclei pushed against the basal pole of the cell. The cells presented different morphologies and considered to be mitochondria-rich cells (*Figure 4*).

In the epithelium lining the posterior part of the accessory gland there are also low cells with flattened nuclei called horizontal cells. The morphological data obtained by Sakai et al. (2012) using the electronic microscopy showed that there are seven types of cells in the whole accessory gland. Some of the cells are secretory cells, others are mitochondria-rich cells, horizontal cells, dark cells and basal cells. In the same study the authors showed that the synthesis and secretion cycle in the accessory gland is long and considered that it is not synchronized with the secretory cycle in the main gland. The secretion of the accessory gland is considered to be small compared with that of the main gland and thus does not contribute in a significantly manner to the whole venom (Mackessy and Baxter, 2006; Sakai et al., 2012).

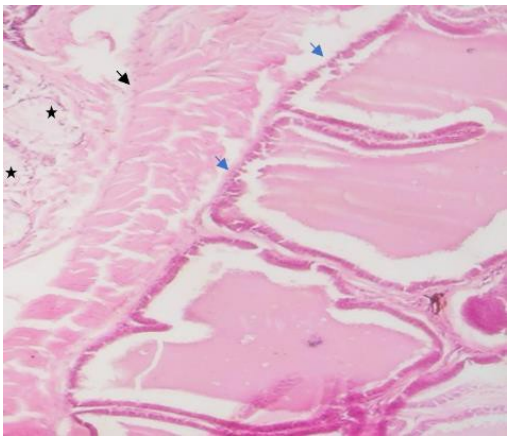

Figure 1. The venom gland (blue arrows), compressor striated muscle fibers (black arrow), mucous acini (asterix). HE. x200

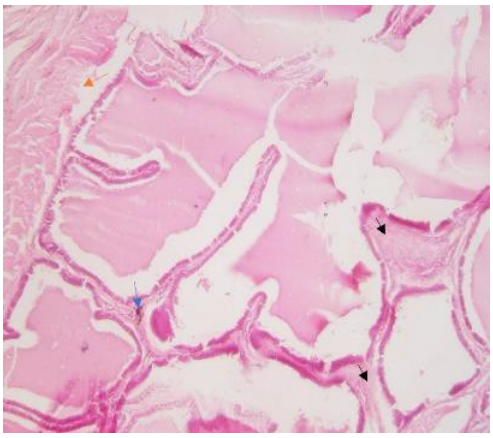

Figure 2. Secretory tubules and connective tissue strands (black arrows) with melanocytes (blue arrow) in venom gland. Skeletal muscle fibers (orange arrow) involved in venom delivery. Venom as a PAS positive secretion. PAS. x100

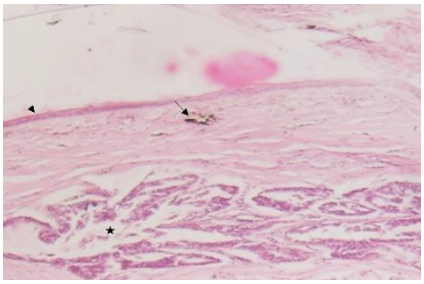

Figure 3. The accessory gland (asterix) situated in the connective tissue containing melanocytes (black arrow) under the skin (arrow head). PAS. X100.

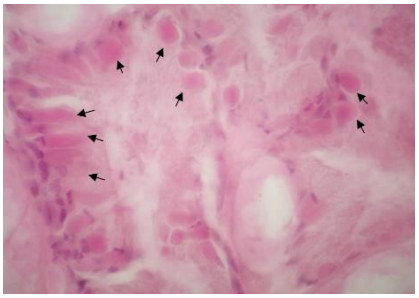

Figure 4. The posterior part of the accessory gland. Different types of mitochondria-rich cells (arrows). PAS. X900

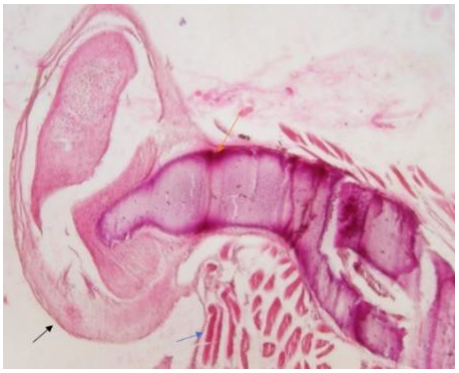

Figure 5. The oral mucosa (black arrow) is folded and covers the venom fang (orange arrow) in the relaxed state. Striated muscle fibres (blue arrow) around the venom fang can be observed. HE. X100

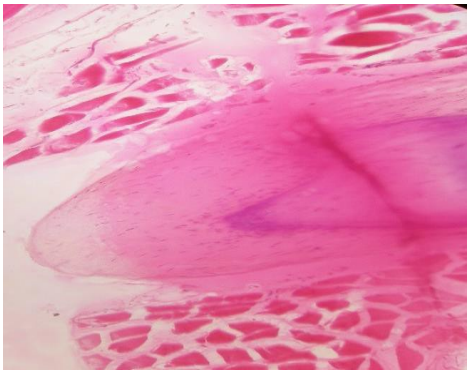

Figure 6. Venom fang and skeletal muscle fibres. PAS, x200.

The anterior part of the accessory gland presented secretory cells which showed different morphological aspects. The cells showed light stained cytoplasm, and displayed round or flattened nuclei. The mucus produced by these cells is considered to be involved in maintaining the moisture, lubrication and reducing the mechanical friction during the strike (Kılıç et al, 2016).

The mucus secretory cells were also observed in the secondary duct that makes the connection between the accessory gland and the fang. These cells showed a PAS positive cytoplasm like the secretion stored in the lumen of this duct. The mucus secretory cells in the secondary duct of other Viperidae were described as having the appearance of the goblet cells present in the gastrointestinal tract and mucus glands of amphibian skin (Kılıç et al., 2016). The studies concerned with the phylogeny of the glands and venom apparatus revealed the importance of the different mucus secretory cells in the oral glands that developed to facilitate the envenoming and swallowing of whole prey (Weinstein et al., 2010). The structural complexity of the accessory

gland does not sustain the hypothesis that this is an evolutionary remnant (Mackessy and Baxter, 2006).

The role of the accessory gland is controversial. It was first considered to be an internal reservoir for the secretory product of the main gland (Kardong K.V., 2002). Other studies suggested that the secretion of the cells in the accessory gland has the role to increase the toxicity of the venom being a source of toxins or the site where the toxins in the venom are activated (Gennaro et al., 2007).

The secretion of both the main and accessory glands is released due to the contraction of the skeletal compressor muscles seen in cross and oblique sections in *Figure 1* and *Figure 2*. In the area around the main gland there were also observed fibers and cells of the connective tissue that accompanied blood vessels that run parallel with the duct.

The venom fang was cut in the relaxed state and laid folded back parallel to the jaw. A fold of mucosa covered the fang that presented an internal canal for venom conduction (*Figure 5*). In a longitudinal section there were also observed numerous skeletal muscle fibers around the fang (*Figure 6*). The fangs are placed on a moveable maxilla and the muscle fibers are involved in rotating it during the strike (Weistein et al., 2010).

Conclussions

The venom apparatus consisted of a main gland that produced the bulk of the venom, a primary duct, an accessory gland and a secondary duct that connected the gland with the fang. The main gland presented secretory units lined with a cuboidal epithelium and venom stored in the lumen. The accessory gland presented two parts: a posterior one with several types of cells and an anterior one with mucous secreting cells. There were no differences observed between the male and female venom gland.

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