



Cardiotoxicity of *Naja nubiae* snakebites: A review and consideration

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Abstract

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Snakebite is a worldwide health problem that threatens the life of the human being. Although the majority of snake species are non-venomous and typically kill their prey with constriction rather than venom, venomous snakes can be found on every continent except Antarctica. Venomous snakes belong to five families: Elapidae, Crotalidae, Viperidae, Hydrophidae and Colubridae, and are capable of producing venom which is used primarily for immobilizing prey and defense mostly via mechanical injection by special fangs. The venoms contain a vast number of substances with different biochemical and pharmacological activities. Cardiotoxins (CTXs), a group of major venom polypeptides of around 60 amino acid residues present abundantly in the elapid snakes, show pharmacological functions including hemolysis, cytotoxicity and depolarization of muscles. Cardiotoxins are the most abundant toxin components of the venoms of cobra snakes. Additionally, cobra cardiotoxin induce severe tissue necrosis and systolic heart arrest in snakebite.

Keywords: Snakebite; Cardiotoxins; Snake venoms.

1. Introduction

Snakebite is a worldwide health problem that threatens the life of the human being. Although the majority of snake species are non-venomous and typically kill their prey with constriction rather than venom, venomous snakes can be found on every continent except Antarctica (**Kasturiratne et al., 2008**). Venomous snakes belong to five families: Elapidae, Crotalidae, Viperidae, Hydrophidae and Colubridae, and are capable of producing venom which is used primarily for immobilizing prey and defense mostly via mechanical injection by special fangs. The venoms

contain a vast number of substances with different biochemical and pharmacological activities (**Tambourgi et al., 1994**). The Nubian spitting cobra is a new species that for long time was considered to be subspecies of *Naja pallida*, but it was separated due to morphological, through its throat and neck pattern. In envenoming, bites from the *Naja nubiae* species are commonly associated with local tissue damages, which could be attributed to the svPLA2 and cytotoxins present in these venoms (Petras et al., 2011). Previous studies also indicated that cobra SVPLA2 enzymes worked

synergistically in case of African spitting cobras with cytotoxins (cardiotoxins) to enhance venom toxicity (Bougis et al.; Tan and Armugam, 1990). Snake venoms are secretion of venomous snake which are synthesized and which are stored in venomous gland. The glands which secrete the zootoxin is a modification of the parotid salivary gland and are situated on each side of head below and behind the eye encapsulated in muscular sheath. Snake venom is a combination of many different proteins, peptides and enzymes and they are generally not dangerous when ingested. Snake venoms are complex mixture of enzymatic and toxic proteins, which include phospholipase A2 (Leon *et al.*, 2011),

Cardiotoxins (CTXs), a group of major venom polypeptides of around 60 amino acid residues present abundantly in the elapid snakes, show pharmacological functions including hemolysis, cytotoxicity and depolarization of muscles (Dufton and Hider, 1988). Cardiotoxins are the most abundant toxin components of the venoms of cobra snakes (Jeyaseelan *et al.*, 1998). Wang *et al.* added that cobra cardiotoxin induce severe tissue necrosis and systolic heart arrest in snakebite victims (Wang *et al.*, 2006).

Review of literature

Cobra venom cardiotoxins (CVCs), a PLA2s, myotoxins, hemorrhagic metalloproteinases and other proteolytic enzymes, coagulant components, neurotoxins, cytotoxins and cardiotoxins (Kini, 2003; Iso known as cardiotoxins, are basic amphipathic proteins that contain a rigid spatial structure, including anti-parallel β -sheets (stabilized by four conserved disulfide bonds) and three elongated finger-like loops, each with polar tips that are flanked by small stretches of highly conserved positively charged Lys and Arg residues (figure 1) (Gasnov, 2014). In addition, cardiotoxins have been classified as either S-type cytotoxins (contain Ser in residue position 28 of loop II) or as P-type cytotoxins (contain Pro in residue position 30 of loop II) (figure 1) (Dubovskii *et al.*, 2005). The molecular mechanisms by which CVCs promote cellular pathology remain unclear. Some evidence has shown that cytotoxins can target the membranes of intracellular organelles, such as lysosomes (Wu *et al.*, 2013), and mitochondria (C.-H. Wang and Wu, 2005). For instance, *Naja* cardiotoxin 3 (CTX3) has been shown to target mitochondria to induce oxidative stress, leading to a collapse of the

mitochondrial transmembrane potential, release of cytochrome C, and activation of apoptosis (Chen *et al.*, 2008).

Myocardial infarction (MI), also known as "heart attack," is the death of cardiac muscle resulting from ischemia. It is by far the most important form of ischemic heart diseases and alone is the leading cause of death in the United States and industrialized nations. About 1.5 million individuals in the United States suffer an acute MI annually and approximately one third of them die. At least 250,000 people a year die of a heart attack before they reach the hospital (Ashley & Niebauer, 2004).

Lipid peroxidation and excessive production of reactive oxygen species (ROS), such as superoxide anions ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), play a major role in the pathogenesis of MI during oxygen shortage. ROS directly injure the cell membrane and cause cell death. ROS also stimulate signal transduction to upregulate inflammatory cytokines, e.g. tumor necrosis factor- α (TNF- α) in the ischemic region and surrounding myocardium. (Miyao *et al.*, 1999)

Myocardial infarction (MI) can be recognized by clinical features, including elevated values of biochemical markers (such as cTn or the MB fraction of creatine kinase (CK-MB), electrocardiographic (ECG) findings, and by imaging (Thygesen *et al.*, 2012).

Myocardial cell death can be recognized by the appearance in the blood of different proteins released into the circulation from the damaged myocytes including myoglobin, cardiac troponin T and I, CK, CK-MB, LDH, as well as many others. Myocardial infarction is diagnosed when blood levels of sensitive and specific biomarkers such as cardiac troponin or CK-MB are increased in the clinical setting of acute myocardial ischemia. Troponins and CK-MB appear in blood within 6 hrs of infarction, (Tn stay 10 days, CK-MB returns within 48 hours (Figure 2) (Miller *et al.*, 2004).

Nearly three-fourths of patients have one or more complications following acute MI, which include the following (Jones *et al.*, 2011):

A- Contractile dysfunction: Myocardial infarcts produce abnormalities in left ventricular function approximately proportional to their size. Most often, there is some degree of left ventricular

failure with hypotension, which may progress to pulmonary edema with respiratory impairment. Severe "pump failure" (cardiogenic shock) occurs in 10% to 15% of patients following acute MI, generally with a large infarct (often greater than 40% of the left ventricle).

B- Arrhythmias: Many patients have conduction disturbances and myocardial irritability following MI, which undoubtedly are responsible for many of the sudden deaths.

C- Myocardial rupture: The cardiac rupture syndromes result from the mechanical weakening that occurs in necrotic and subsequently inflamed myocardium and include (1) rupture of the ventricular free wall (most commonly), (2) rupture of the ventricular septum (less commonly), leading to a left-to-right shunt and (3) papillary muscle rupture (least commonly).

Myocarditis is an inflammatory disease of the muscular tissues of heart that may present with sudden cardiac death, symptoms resembling myocardial infarction, heart rhythm and conduction disorders, and heart failure. Infectious, mostly viral, auto-immune and toxic agents are the main etiologies for the pathogenesis of myocarditis. The incidence of myocarditis is largely unknown

because of difficulties establishing the diagnosis and the lack of a gold standard other than myocardial biopsy (Maron et al., 2009). Snake bites have been -among other factors- implicated in inducing an autoimmune reaction in the heart that is often characterized by acute rash, fever, peripheral eosinophilia, and ECG abnormalities such as nonspecific ST segment changes or infarct patterns. However, some patients present with sudden death or rapidly progressive Heart Failure. The true incidence is unknown, but in some series, the prevalence of clinically undetected HSM in explanted hearts ranged from 2.4 to 7 percent (Sheikh et al. 2018). Myocarditis of this type is usually associated with hyper eosinophilia that it may be called eosinophilic myocarditis (EM). Endomyocardial biopsy is considered to be a gold standard for the diagnosis of EM. Echocardiography, cardiac magnetic resonance, and bio markers particularly serum eosinophilic cationic protein concentrations are also known to aid in diagnosis. EM may lead to progressive, irreversible, and fatal myocardial damage if prompt diagnosis is not made and therapy is not initiated. The current treatment regimens include corticosteroids, cytotoxic agents, and immunosuppressive therapy. However, a proper treatment criterion is yet to be established (Kindermann et al. 2012).

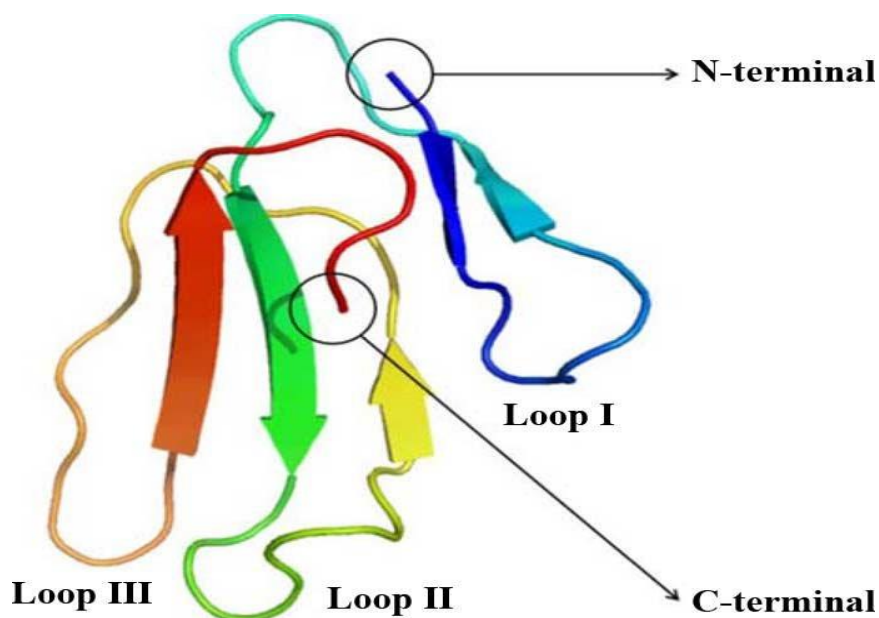


Figure 1: Structure of the cobra cardiotoxin (CTX) protein

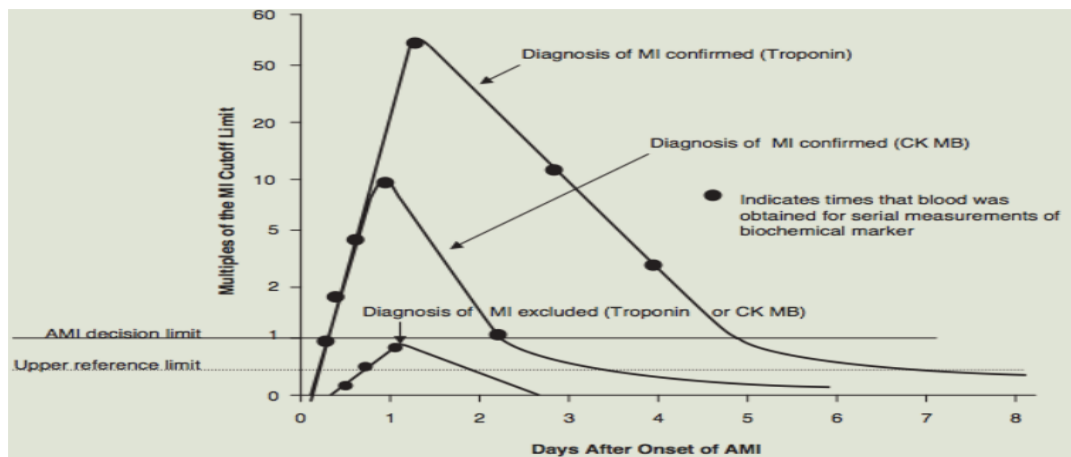


Figure 2: Changes in cardiac markers with time in AMI

The gold standard in diagnosis of myocarditis is still the myocardial biopsy. Acute myocarditis is defined by lymphocytic infiltrates in association with myocyte necrosis. While borderline myocarditis is characterized by inflammatory infiltrates without evidence of myocyte necrosis (Aretz., 1987). This technique is limited by the high variability in interpreting biopsy specimens (in particular with regard to borderline myocarditis) and because non-cellular inflammatory processes cannot be detected. Thus, immunohistochemistry is gaining further acceptance in the diagnosis of myocarditis. Monoclonal antibodies allow the characterization and localization of the mononuclear cell infiltrates (Pawlak and Gil, 2017).

Autopsy results of myocardial tissues with myocarditis usually demonstrate increased cardiac weight, intramyocardial extravasation of blood, and fibrin or fibrin-platelet microthrombi in capillaries. Features in keeping with myocardial necrosis on electron microscopy include hypercontraction bands, myofibrillar damage, lysis, intra-mitochondrial electron dense inclusions, and fibrin deposition in myocyte cytoplasm.

Several subsequent gross pathological examinations have confirmed that myocardial edema, thickening of the left ventricular wall and interventricular septum, myocardial necrosis, pericardial effusions, and fibrinous pericarditis may present in such cases which subsequently lead to severe cardiac toxicity and dilated cardiomyopathies as can be seen in figure 3 (Yeh et al. 2004).

Future recommendation:

Thymoquinone, a bioactive molecule with wide range of promising effects is known for being both antioxidant and cardioprotective in diverse studies in literature. Studying its protective effect against snake crude venom and elucidating a possible protective pathway could open a new way in the process of alleviating *Naja nubiae* known cardiotoxicity. It was demonstrated that TQ possessed promising cardioprotective effects in isoproterenol-induced myocardial injury in rats. Isoproterenol is known to induce myocardial lesions similar to acute myocardial infarction in rats. TQ treatment augmented anti-oxidant activity and salvaged cardiomyocytes evidenced by restoration of cardiac injury enzymes and reduction of lipid peroxidation product and pro-inflammatory cytokines. The findings were further supported by histopathological preservation of the myocardium as evidenced by reduced myonecrosis, edema, and infiltration of inflammatory cells. It was revealed that TQ exert protective effect on cardiac injury by attenuating oxidative stress, enhancing endogenous anti-oxidants and maintaining structural integrity (Ojha et al., 2015). Thymoquinone supplementation could take part in the antivenom treatment regimens to protect against venom-induced cardiotoxicity in individuals living near or where *Naja nubiae* species inhabit in areas of upper Egypt. In this context, people living in those area can be encouraged to cultivate and implement proportions of black cumin seeds in their food regimes benefiting from TQ discovered effects of cardioprotection.

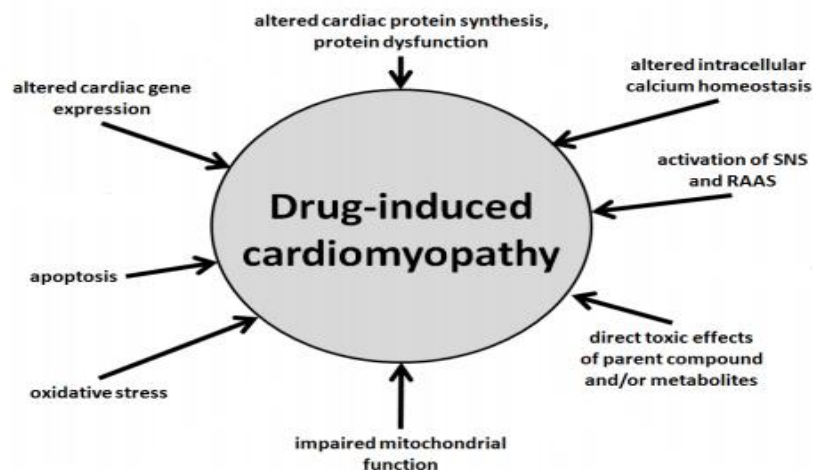


Figure 3: Different pathogenesis of myocarditis and subsequent DCM

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