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Mini review

Matrix biology meets toxinology

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ABSTRACT

Venoms are cocktails containing pharmacologically active compounds, which drastically affect essential functions of the neuromuscular and cardiovascular system, as well as of blood, kidney and other organs. As the extracellular matrix and its contacts with cells are responsible for maintaining the integrity and functionality of these organs and tissues, it is not surprising that several venom components target matrix molecules and their respective cellular receptors. Many venom components, such as matrix-degrading enzymes, disintegrins, and C-type lectin-like proteins, have been identified and have laid the foundation for the frontier research field of matrix toxinology. Interestingly, many toxins consist of domains which are structurally homologous to modules and domains of matrix proteins, their proteinases and cellular receptors. In addition to finding new agents and tools, which specifically interact with matrix molecules and their receptors, the characterization of known matrix-targeting toxins will provide insights into their molecular modes of action and thus may lead to potential new therapeutic strategies for treating matrix-related diseases, such as blood clotting and thrombocyte-mediated disorders, but also tumor malignancies.

Contents

1. Toxinology — turning natural toxins into pharmaceuticals

Toxinologists search for naturally occurring toxins in animals, plants and microorganisms (see also homepage of the International Society of Toxinology (IST) under http://www.toxinology.org). Clinical toxinologists describe the symptoms of envenomation and aim to identify the responsible venom components and their molecular mechanisms. This is to lead to a therapy for an envenomed person. Snakes, spiders, scorpions, jellyfish and other venomous animals still pose a major risk for man. In fact, every year two million people are estimated to be bitten by snakes. Tens of thousands of these victims lose their lives (Kasturiratne et al., 2008). The high morbidity and mortality affect people especially in rural areas in the tropics and subtropics. After envenomation, the victims need immediate medical help, which nowadays consists mainly of an antibody-based antivenom therapy, if available, and in the treatment of systemic and local symptoms (Gutiérrez et al., 2007). Natural venoms usually are cocktails of pharmacologically highly effective components, each of which is directed

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against different target molecules and thus affects various tissues and organ systems, such as the nervous system, the cardiovascular system, kidneys, and muscles (De Lima et al., 2009; Mackessy, 2009; Mebs, 2002). Therefore, toxinologists also try to use purified venom components to treat diseases which are not caused by envenomation and are non-related to bites and stings of venomous animals. Whereas the entire venom mixture is detrimental to an organism, single constituents of venoms can be medically very helpful, as they are evolutionarily adapted to bind their target molecules selectively and effectively, two prerequisites for a successful pharmacon (Koh et al., 2006). Therefore, learning about the components of animal venoms may not only help to treat envenomed victims successfully, but also bears the great chance to serve as a platform to discover new drugs and to open novel avenues to treat common and severe diseases related to matrix, *e.g.* thrombosis, stroke, nerve degeneration, and heart disorders (Calvete 2009; Escoubas and King, 2009; Ménez et al., 2006).

But who thinks of matrix biology when facing a snake, a blood-sucking insect, a leech, or another venomous animals? Nonetheless, natural venoms have quite a bit to offer to scientists who aim to characterize extracellular matrix components, their interplay within the shape-forming scaffold and their contacts with cells. Many symptoms of envenomation, such as local blister formation and systemic disorders of hemostasis, phenocopy genetic or acquired deficiencies of matrix molecules. This lays the foundation for a frontier research field of "matrix toxinology", which not only promise a gain in scientific knowledge but also pinpoints new pharmacological strategies to treat matrix-involved diseases. Although one might consider this new research field and its expected results revolutionary, it is not unprecedented. For the last decades, many toxinologists have focused on venom components, which target ion channels. α-Bungarotoxin from the krait snake venom (*Bungarus multicinctus*) was instrumental in identifying and characterizing the ligand-gated sodium channel, named the muscle type nicotinic acetylcholinergic receptor (nAChR), and its physiological functions (Albuquerque et al., 2009), as witnessed by almost 4800 publications since its discovery in 1970. Other snake venoms, such as the α-cobratoxins, and the α-conotoxins from the venoms of the marine cone snails, also target nAChR, resulting in a fast paralysis of the predator or prey. Beyond their usage in basic research to identify ion channels, the sea anemone toxin anthopleurin-A, the ω-conotoxins from the cone shell venom and other neurotoxins from various animals have emerged as potential analgetics (Bailey and Wilce, 2001; Han et al., 2008; Koh et al., 2006; Stolarz Oliveira et al., 2009). Anthopleurin-A is directed against the voltage-gated sodium channel, and therefore is a candidate compound to clinically manipulate muscle tonus and cardiac functions (El-Sheriff and Turitto, 2003). From the venoms of cone snails, several groups of conotoxins have been isolated. These peptides and small proteins are mainly directed against ion channels (Becker and Terlau, 2008; Olivera, 2006; Terlau and Olivera, 2004). Of major medical importance, the ω-conotoxins, about 40 amino acids long, target neuronal voltage-gated calcium channels of dorsal ganglions, which are involved in pain perception (Terlau and Olivera, 2004). After clinical trials showed that the ω-conotoxin ω-MVIIA from *Conus magus* is more effective than morphin in blocking the nocireceptive receptors, it is now clinically used as analgesic named Ziconitide (Prialt®) (Han et al., 2008). The venoms of other marine venomous animals (Watters, 2005), such as sea snakes, fish (Gomes de Figueiredo et al., 2009) and jellyfish (Brinkman and Burnell, 2009; Tibballs, 2006) are under investigation for their potential to treat neuronal and cardiovascular disorders.

In addition to ion channel blockers, potent enzymatic inhibitors were identified from snake venoms. Blocking the angiotensin-converting enzyme (ACE), the originally called bradykinin-potentiating peptide from the *Bothrops jararaca* venom causes a drastic drop in blood pressure. Its synthetic mimetics, such as captopril, have founded a new generation of ACE inhibitors to treat hypertension (Vane, 1999).

2. Matrix toxinology, an interdisciplinary approach

In comparison to the successes, which toxinologists accomplished together with ion channel researchers, the joint research field of matrix toxinology is still in its infancy. This review reports where matrix toxinology stands today and how it might develop. Neglecting the myriad of naturally occurring venoms in plants and microorganisms, I focus on animal venoms to be able to provide some examples in detail.

What can animal venoms offer to matrix biology? The best examined animal venoms are from snakes. Several snake species have thoroughly been investigated by proteomics, an approach known as 'venomics' (Calvete et al., 2009b; Fox and Serrano, 2008; Gutiérrez et al., 2009). Most of the snake venom constituents act hemorraghically or neurotoxically (Yamazaki and Morita, 2007). Whereas snakes from the elapid species mainly cause neuropathologies and paralysis, the most detrimental effects of envenomation by colubrid species are local tissue necrosis, failure of blood clotting and hypofibrinogenemia, resulting in severe and lethal bleedings (Calvete et al., 2009). Comprising hundreds of additional pharmacologically active compounds, snake venoms target not only the humoral blood clotting system and ion channels on neurons and muscle cells, but also almost any organ system of the body, such as the cardiovascular system, kidney, and connective tissue (Fig. 1). Although investigations have been intensified, many venom components, including their respective targets within the victim's organism and their molecular mode of action, still remain undiscovered. Using an appropriate molecular target, *e.g.* a recombinantly tagged matrix molecule or its receptor, as bait (Eble et al., 2001, 2003), matrix biologists can search for a relevant binding/inhibitory toxin by bioassay-guided fractionation of the crude venom (Escoubas and King, 2009). Alternatively, the most recent 'venomics' approach is large scale identification of all venom components by using proteomics and cDNA libraries of the venoms and venom glands, respectively (Calvete, 2009; Escoubas and King, 2009; Fox and Serrano, 2008; Ménez et al., 2006). After identification, the venom components are characterized for their effects on matrix molecules or as agonist or antagonist of a respective matrix–cell-interaction. Thereafter, it may be a valuable tool not only to gain insights into its mechanisms at the molecular and cellular level but also may serve as a lead structure to develop highly effective drugs (Calvete, 2009; Escoubas and King, 2009; Koh et al., 2006). Two extensively scrutinized "classical" targets of venom components are well known in the research field of matrix biology: (i) fibrinogen as the typical matrix molecule of the blood, and (ii) cell–matrix interactions. Both examples are described in more detail.

3. Fibrinogen as a toxinologic target is a contact point between matrix biology and toxinology

A common organ system which is targeted by venoms is the blood. Clotting converts fluid blood within minutes into a solid matrix structure, mainly composed of the network of insoluble fibrin molecules generated from the blood protein fibrinogen (Weisel, 2005). Fibrinogen consists of an N-terminally connected pair of heterotrimers, each made up from an Aα, Bβ and γ chain (Mosesson et al., 2001; Weisel, 2005). The γ chain comes in two isoforms, γ and γ′, with a natural occurrence of 92% and 8%, respectively. The two alterative splice variants γ and γ′ differ in their C-termini which harbor a cell interaction site and an anionic antithrombin I binding site, respectively (Fig. 2).

In a multistep process, which includes both proteolytic cleavages and consequential conformational changes, fibrinogen is converted into fibrin (Fig. 2), which forms the highly ordered scaffold that is typical of extracellular matrix (ECM) proteins (Mosesson et al., 2001). In the first step, activated thrombin removes the fibrinopeptide A (FPA, 16 amino acids long) from the Aα chain, thereby exposing the