FEBS Letters 583 (2009) 1736–1743

journal homepage: www.FEBSLetters.org

Minireview

Venoms, venomics, antivenomics

Juan J. Calvete [a,*], Libia Sanz [a], Yamileth Angulo [b], Bruno Lomonte [b], José María Gutiérrez [b]

[a] Instituto de Biomedicina de Valencia, C.S.I.C., Jaume Roig 11, 46010 Valencia, Spain
[b] Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica

ARTICLE INFO

Article history:
Received 17 February 2009
Revised 12 March 2009
Accepted 13 March 2009
Available online 20 March 2009

Edited by Miguel De la Rosa

Keywords:
Proteomics of snake venom
Snake venomics
Snake venom
Antivenom
Antivenomics

ABSTRACT

Venoms comprise mixtures of peptides and proteins tailored by Natural Selection to act on vital systems of the prey or victim. Here we review our proteomic protocols for uncoiling the composition, immunological profile, and evolution of snake venoms. Our long-term goal is to gain a deep insight of all viperid venom proteomes. Knowledge of the inter- and intraspecies ontogenetic, individual, and geographic venom variability has applied importance for the design of immunization protocols aimed at producing more effective polyspecific antivenoms. A practical consequence of assessing the cross-reactivity of heterologous antivenoms is the possibility of circumventing the restricted availability of species-specific antivenoms in some regions. Further, the high degree of target specificity makes toxins valuable scaffolds for drug development.

1. The Ying and Yang of animal venoms

Venomous organisms are widely spread throughout the animal kingdom, comprising more than 100,000 species distributed among all major phyla, such as chordates (reptiles, fishes, amphibians, mammals), echinoderms (starfishes, sea urchins), molluscs (cone snails, octopi), annelids (leeches), nemertines, arthropods (arachnids, insects, myriapods) and cnidarians (sea anemones, jellyfish, corals). In any habitat there is a competition for resources, and every ecosystem on Earth supporting life contains poisonous or venomous organisms. One of the most fascinating techniques of capturing prey or defending oneself is the use of poisons or venoms. Venom represents an adaptive trait and an example of convergent evolution [1–3]. Venoms are deadly cocktails, each comprising unique mixtures of peptides and proteins naturally tailored by Natural Selection to act on vital systems of the prey or victim. Venom toxins disturb the activity of critical enzymes, receptors, or ion channels, thus disarranging the central and peripheral nervous systems, the cardiovascular and the neuromuscular systems, blood coagulation and homeostasis. On the other hand, due to their high degree of target specificity, venom toxins have been increasingly used as pharmacological tools and as prototypes for drug development. The medicinal value of venoms has been known from ancient times. The snake is a symbol of medicine due to its association with Asclepius, the Greek god of medicine. The medical uses of scorpion and snake venoms are well documented in folk remedies, and in Western and Chinese traditional medicine [4,5]. However, extensive investigations on venom compounds as natural leads for the generation of pharmaceutical products have only been performed in the last decades, after a bradykinin-potentiating peptide isolated from the venom of the Brazilian viper *Bothrops jararaca* was developed in the 1950s into the first commercial angiotensin I-converting enzyme (ACE)-inhibiting drug, captopril®, for the treatment of renovascular hypertension [6,7]. The latest example of development of a toxin into an approved drug by the US Food and Drug Administration (FDA) (December 2004) is ziconotide (Prialt®), a synthetic non-opioid, non-NSAID, non-local anesthetic drug originating from the cone snail *Conus magus* peptide ω-conotoxin M-VII-A, an N-type calcium channel blocker. Discovered in the early 1980s [8], zicotonide is a rare example of a molecule used unaltered from a creature's chemistry. Prialt® is used for the amelioration of chronic untreatable pain, and due to the profound side effects or lack of efficacy when delivered through more common routes, such as orally or intravenously, Prialt® must be administered directly into the spine.

Venoms represent a huge and essentially unexplored reservoir of bioactive components that may cure disease conditions which do not respond to currently available therapies. The great pharmacological cornucopia accumulated by Nature over evolution has resulted in true combinatorial libraries of hundreds of thousands of potentially active and useful molecules synthesised in the venoms of the 700 or so known species of Conus, the roughly 725 species of

* Corresponding author. Fax: +34 96 369 0800.
E-mail address: jcalvete@ibv.csic.es (J.J. Calvete).

0014-5793/$36.00
doi:10.1016/j.febslet.2009.03.029

venomous snakes, the ~1500 species of scorpions, or the ca. 37,000 known species of spiders, just to cite only a few examples of venomous animals. The accelerated Darwinian evolution acting to promote high levels of variation in venom proteins may be part of a predator–prey arms race that allows the predator to adapt to a variety of different prey, each of which are most efficiently subdued with a different venom formulation. In addition to understanding how venoms evolve, a major aim of venomic projects is to gain a deeper insight into the spectrum of medically important toxins in venoms to uncover clues in order to solve the riddle of the medical effectiveness of venoms and to learn how to convert deadly toxins into lifesaving drugs [9,10]. On the other hand, envenomation constitutes a highly relevant public health issue on a global basis, as there are venomous organisms in every continent and almost every country. However, venomous animals are particularly abundant in tropical regions, which represent the kitchen of evolution. Arthropod stings inflicted by bees, wasps, ants, spiders, scorpions, and – to a lesser extent – millipedes and centipedes, constitute the most common cause of envenoming by animals, although around 80% of deaths by envenomation worldwide are caused by snakebite, followed by scorpion stings, which cause 15%. Envenoming constitutes a highly relevant public health issue on a global basis, although it has been systematically neglected by health authorities in many parts of the world [11–15]. Being a pathology mainly affecting young agricultural workers living in villages far from health care centers in low-income countries of Africa, Asia and Latin America, it must be regarded as a 'neglected tropical pathology' [11]. Adequate treatment of envenoming is critically dependent on the ability of antivenoms to neutralize the lethal toxins reversing thereby the signs of envenoming. A long-term research goal of venomics of applied importance for improving current antivenom therapy is to understand the molecular mechanisms and evolutionary forces that underlie venom variation. Thus, a robust knowledge of venom composition and of the onset of ontogenetic, individual, and geographic venom variability may have an impact in the treatment of bite victims and in the selection of specimens for the generation of improved antidotes [16] (see below).

2. The evolution of the advanced snakes and their venoms

The suborder of snakes (Serpentes) of the reptilian order Squamata, named for their scaly skin, includes about 3000 extant species placed in approximately 400 genera and 18 families (http://www.reptile-database.org). The timing of major events in snake evolution is not well understood, however, owing in part to a relatively patchy and incomplete fossil record [17,18]. Nevertheless, after more than 100 years of research, the most generalized phylogenetic view is that the group evolved from a family of terrestrial lizards during the time of the dinosaurs in the Jurassic period, about 200 million years (Myr) ago [19]. After the end of the non-avian dinosaurs reign, around the Cretaceous-Tertiary boundary 65 Myr ago [20], the boids (the ancestors of boas, pythons and anacondas) were the dominant snake family on Earth. Within the Cenozoic era that followed, advanced snakes (colubrids) arose as long ago as in the Oligocene epoch (35–25 Myr). Colubrids, the family which we regard today as typical snakes, remained a small taxon until the tectonic plates drifted apart from the equator and the cool climate pushed boids to disappear from many ecological niches. Colubrids quickly colonized these empty habitats and this family today comprises over two-thirds of all the living snake species [21]. Colubroidea encompasses Viperidae (30 genera, 230 species of vipers and pitvipers), Elapidae (63 genera, 272 species of corals, mambas, cobras and their relatives), Atractaspididae (14 genera, 65 species of Stiletto snakes and mole vipers), and Colubridae (290 genera, almost 1700 species of rear-fanged and "harmless" colubrids) (http://www.reptile-database.org). Noteworthy, the front-fanged venom-delivery system appeared three times independently in Viperidae, Elapidae, and Atractaspididae [22].

The presence of a venom-secreting oral gland is a shared derived character of the advanced (Caenophidia) snakes. All venomous squamates, snakes and venomous lizards such as gila monster, beaded lizard, komodo dragon, etc., share a common venomous ancestor [1]. Given the central role that diet has played in the adaptive radiation of snakes [23], venom thus represents a key adaptation that has played an important role in the diversification of these animals. Venoms represent the critical innovation in ophidian evolution that allowed advanced snakes to transition from a mechanical (constriction) to a chemical (venom) means of subduing and digesting prey larger than themselves, and as such, venom proteins have multiple functions including immobilizing, paralyzing, killing and digesting prey. Venoms of snakes in the families Viperidae and Elapidae are produced in paired specialized venom glands located in the upper jaw, ventral and posterior to the eyes [24], and introduced deeply into prey tissues via elongate, rotatable fangs. Viperids and elapids possess the most widely studied types of animal toxins [9,25,26]. These snake venoms contain complex mixtures of hundreds of important pharmacologically active molecules, including low molecular mass organic and inorganic components (histamine and other allergens, polyamines, alkaloids), small peptides and proteins [9,27,28]. The biological effects of venoms are complex because different components have distinct actions and may, in addition, act in concert with other venom molecules. The synergistic action of venom proteins may enhance their activities or contribute to the spreading of toxins. According to their major toxic effect in an envenomed animal, snake venoms may be conveniently classified as neurotoxic and haemotoxic. Among the first group are the Elapidae snakes (mambas, cobras, and particularly the Australian snakes, which are well known to be the most toxic in the world). On the other hand, snakes of the family Viperidae (vipers and pitvipers) contain numerous proteins that typically disrupt the function of the coagulation cascade, the haemostatic system and tissue integrity, which are manifested as bleeding and incoagulable blood and local tissue necrosis in human victims of envenoming [9,26,27].

The existence in the same venom of a diversity of proteins of the same family but differing from each other in their pharmacological effects reflects an accelerated positive Darwinian evolution. Venom toxins likely evolved from proteins with a normal physiological function and appear to have been recruited into the venom proteome before the diversification of the advanced snakes, at the base of the Colubroid radiation [1,29,30]. Gene duplication followed by functional divergence is the main source of molecular novelty. Gene duplication creates redundancy and allows a gene copy to be selectively expressed in the venom gland, escaping the pressure of negative selection and evolving a new function through positive selection and adaptative molecular evolution [31–33]. The occurrence of multiple isoforms within each major toxin family evidences the emergence of paralogous groups of multigene families across taxonomic lineages where gene duplication events occurred prior to their divergence, and suggests an important role for balancing selection in maintaining high levels of functional variation in venom proteins within populations. The mechanism leading to this mode of selection is unclear but it has been speculated that it may be related to unpredictability with which a sit-and-wait predator like a rattlesnake encounters different types of prey, each of which are most efficiently subdued with different venom proteins [34,35]. Thus, to deal with this uncertainty, snakes are required to have a variety of proteins "available" in their venom at all times to deal with different prey. The selection pressure leading to high levels of variation in venom genes may parallel the selec-