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Review

Non-enzymatic proteins from snake venoms: A gold mine of pharmacological tools and drug leads

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ABSTRACT

Non-enzymatic proteins from snake venoms play important roles in the immobilization of prey, and include some large and well-recognized families of toxins. The study of such proteins has expanded not only our understanding of venom toxicity, but also the knowledge of normal and disease states in human physiology. In many cases their characterization has led to the development of powerful research tools, diagnostic techniques, and pharmaceutical drugs. They have further yielded basic understanding of protein structure–function relationships. Therefore a number of studies on these non-enzymatic proteins had major impact on several life science and medical fields. They have led to life-saving therapeutics, the Nobel prize, and development of molecular scalpels for elucidation of ion channel function, vasoconstriction, complement system activity, platelet aggregation, blood coagulation, signal transduction, and blood pressure regulation. Here, we identify research papers that have had significant impact on the life sciences. We discuss how these findings have changed the course of science, and have also included the personal recollections of the original authors of these studies. We expect that this review will provide impetus for even further exciting research on novel toxins yet to be discovered.

1. Introduction

Snake venoms are complex mixtures of pharmacologically active proteins and polypeptides. Some of these proteins exhibit enzymatic properties, whereas others are considered non-enzymatic. Enzymes contribute toward the lethal and debilitating effects of venom in addition to their putative role in the digestion of prey. Non-enzymatic proteins, on the other hand, contribute mainly toward immobilization of prey. They bind to specific receptors, ion channels or plasma proteins and interfere in the prey's physiological processes; they act as agonists or antagonists and lead to neurotoxic, cardiotoxic and/or tissue necrotizing effects. Over the last few decades, several hundreds of non-enzymatic proteins have been purified and characterized from snake venoms. Based on their amino acid sequences and protein folds, they are classified into several structural or functional protein families. Some of the well-recognized families of non-enzymatic proteins in snake venoms are: (i) three-finger toxins; (ii) proteinase inhibitors; (iii) snaclecs (C-type lectins and related proteins); (iv) nerve growth

Abbreviations: ACEi, angiotensin converting enzyme inhibitor; BPP, bradykinin potentiating peptide; CRISP, cysteine-rich secretory protein; CVF, cobra venom factor; EGF, epidermal growth factor; IX/X-bp, factor IX/X binding protein; NGF, nerve growth factor; NP, natriuretic peptide; PLA_2, phospholipase A_2; SRTX, sarafotoxin; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; vWD, von Willebrand disease; vWF, von Willebrand factor.

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factors; (v) bradykinin-potentiating peptides; (vi) natriuretic peptides; (vii) cysteine-rich secretory proteins (CRISPs) or helveprins; (viii) sarafotoxins; (ix) cobra venom factors; (x) vascular endothelial growth factors; (xi) waprins; (xii) vespryns; and (xiii) veficolins. The proteins within each family share remarkable similarities in their primary, secondary and tertiary structures, but they may differ from each other in their pharmacological effects. Such vivid functional diversity is well documented, particularly among three-finger toxins (Hegde et al., 2009; Kini and Doley, 2010), snaclecs (Du and Clemetson, 2009; Clemetson, 2010a,b) and serine proteinase inhibitors (Župunski et al., 2003; van Gent et al., 2003). Thus, toxin structure–function relationships and mechanisms of actions are intriguing, and they pose exciting challenges.

The studies of snake venoms and toxins have focused on one or more of the following objectives: (i) to determine the mode and mechanism of action of the toxins; (ii) to find ways and means to neutralize the toxicity and adverse effects of snake bite envenomation; (iii) to develop specific research tools that are useful in understanding normal physiological processes at both cellular and molecular levels; and (iv) to develop prototypes of pharmaceutical agents based on the structures of toxins (Kini, 2002). Important lessons can be learned, particularly from the latter two objectives, as to how simple molecular templates have been used in nature to design a wide arsenal of proteins that exhibit diverse biologic functions. Over the last three or four decades—due to the efforts of scientists from various backgrounds including biology, protein chemistry, molecular biology, pharmacology, toxinology, and structural biology—significant progress has been made in understanding the structure–function relationships and mechanisms of action of a number of non-enzymatic proteins. These studies have contributed significantly to the development of research tools and have provided new therapeutic agents. To achieve these aforementioned goals, a number of key papers were pivotal in making significant progress in their respective fields. The papers that have contributed to our understanding of three-finger toxins (Utkin, 2013) and disintegrins (Calvete, 2013) have been reviewed separately. Here, we will attempt to identify some of the papers that have made major contributions to the understanding of various non-enzymatic proteins and discuss their contribution to snake venom toxinology.

2. Key advances in the research on non-enzymatic proteins

2.1. Paradigm shift – toxins in developing therapeutics to save millions of lives

Snakes and their venoms, at first glance, appear to be "villains"; they kill or maim thousands of healthy individuals every year, even in the 21st century, in tropical countries (Alirol et al., 2010). Snake venom toxins can cause undesirable effects in victims, from debilitation to death. The toxicity of these proteins is one of the main reasons for our fascination with snakes, and for centuries scientists have focused on understanding this toxicity and the ways to neutralize it. The discovery of bradykinin-potentiating peptides (BPPs) expanded this view when it provided the basis for the development of life-saving, anti-hypertensive drugs including Captopril and Enalapril (Fig. 1). To our knowledge, this was the first instance in which the

Fig. 1. Examples of the pharmaceutical drugs developed based on the activity of bradykinin potentiating peptide. Ramipril (tajpharma.com), Captopril (everestconsultores.com), Enalapril (magistracc.com), Lisinopril (blurx.us), and Perindopril (electromedias.net).