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Review

Pathophysiological significance and therapeutic applications of snake venom protease inhibitors

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ABSTRACT

Protease inhibitors are important constituents of snake venom and play important roles in the pathophysiology of snakebite. Recently, research on snake venom protease inhibitors has provided valuable information to decipher the molecular details of various biological processes and offer insight for the development of some therapeutically important molecules from snake venom. The process of blood coagulation and fibrinolysis, in addition to affecting platelet function, are well known as the major targets of several snake venom protease inhibitors. This review summarizes the structure-functional aspects of snake venom protease inhibitors that have been described to date. Because diverse biological functions have been demonstrated by protease inhibitors, a comparative overview of their pharmacological and pathophysiological properties is also highlighted. In addition, since most snake venom protease inhibitors are non-toxic on their own, this review evaluates the different roles of individual protease inhibitors that could lead to the identification of drug candidates and diagnostic molecules.

Contents

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1. Introduction

Protease inhibitors are an important class of proteins or polypeptides that inhibit the catalytic activity of proteolytic enzymes. The molecules are ubiquitous, exist in multiple forms, and are produced by several tissues of animals and plants and by microbes to perform specialized function(s) for the producing organism (Mourão and Schwartz, 2013). A typical mammalian genome is estimated to contain approximately 2–4% of its genes encoding for proteases and/or protease inhibitors, indicating the significance of proteolysis and the inhibition of protease activity in the cell's metabolic activity (Puente et al., 2005). Over the past few decades, numerous protease inhibitors have been isolated, purified, and characterized (López-Otín and Overall, 2002; Mourão and Schwartz, 2013). According to the class of proteases being inhibited, protease inhibitors are classified as either: serine, cysteine, aspartic, or metallo (Abbenante and Fairlie, 2005; Mourão and Schwartz, 2013).

Snake venom is a cocktail of bioactive components including proteases and protease inhibitors (Matsui et al., 2000; Krowarsch et al., 2003; Kini, 2003). Protease inhibitors were initially reported to be in snake venom by Takahashi et al. (1972), who isolated a 7 kDa peptide from *Daboia russelii* venom that was a potent inhibitor of kallikrein, plasmin, and trypsin. Since then, a tremendous interest has been shown to identify and characterize Kunitz-type serine protease inhibitors (KSPIs) from the venom of several other species of snakes. The amino acid and/or cDNA sequences of several KSPIs has been determined and some of their biological functions have been elucidated (Morjen et al., 2013; Guo et al., 2013a, 2013b; Mourão and Schwartz, 2013; Mukherjee et al., 2014a, 2016; Mukherjee and Mackessy, 2014). Besides the KSPIs, several other enzymatic and non-enzymatic components of snake venom, such as phospholipase A_2 (PLA_2) and C-type lectin-like proteins (snaclec) were subsequently shown to inhibit serine proteases by non-enzymatic mechanisms. The pharmacological effect inhibiting the serine proteases of the host is important for inflicting the venom-induced toxicity (Clemetson et al., 2009; Clemetson, 2010; Osipov et al., 2010; Mukherjee et al., 2014a, 2014b, 2014c, 2016). In recent decades, a number of interesting review articles have been published on the structure-function properties of many snake venom toxins. Still, gaps exist in our knowledge about the pathophysiological or pharmacological effects of protease inhibitors in victims or prey, and little is known about possible biomedical applications for the biomolecules derived from snake venom. This review article presents an updated overview of the structure-functional properties, pathophysiological significance, and possible therapeutic applications of protease inhibitors from snake venom.

2. Classification of snake venom protease inhibitors based on their structure-functional properties

Among the different classes of protease inhibitors characterized to date, the serine group is the most extensively distributed superfamily of protease inhibitors found in snake venom (Cardle and Dufton, 1997; Krowarsch et al., 2003; Župunski et al., 2003; Mourão and Schwartz, 2013; Ranasinghe and McManus, 2013). In contrast, only a few cysteine protease inhibitors have been reported from snake venom (Mashiko and Takahashi, 2002), and while one matrix metalloprotease inhibitor has been reported (Inagaki et al., 2012), aspartic inhibitors have yet to be documented in snake venom.

2.1. Kunitz-type serine protease inhibitors

The Kunitz-type serine protease inhibitors (KSPIs) are ubiquitous. They likely contain 58-60 amino acid residues including three disulfide bridges, and an antiproteinase site (P1 site) that is responsible for their specificity to their cognate enzymes (Župunski et al., 2003; Guo et al., 2013a, 2013b; Mukherjee et al., 2014a). In most of the canonical small inhibitors, like the KSPIs, the intermolecular interactions of the subsites result in elongation on both sides of the scissile bond of the P1 site of the KSPI, a vital prerequisite for inhibition of serine proteases (Bode and Huber, 1992). Laskowski and Kato (1980) suggested that a positively charged residue (Arg or Lys) occupies the P1 site of the trypsin inhibitor; whereas, a large hydrophobic residue (such as Leu, Phe, or Tyr) is typically found in the P1 site of the chymotrypsin inhibitor (Laskowski and Kato, 1980). Therefore, the residue in the P1 site of a KSPI is the key determinant of the inhibitor's specificity towards a specific serine protease substrate (Laskowski and Kato, 1980).

Structurally, snake venom KSPIs resemble bovine pancreatic trypsin inhibitor (BPTI), which exemplifies the Kunitz inhibitors. The sequences of snake venom KSPIs are compared with that of BPTI in Table 1. In the KSPIs, two disulphide bonds between C1 and C6, and C3 and C5 are necessary for the conservation of their intrinsic conformation and a third disulphide bond (between C2 and C4) is necessary to stabilize the two protease-binding domains of KSPIs (Laskowski and Kato, 1980). Closer sequence analyses of snake venom KSPIs have demonstrated the occurrence of conserved residues at the core and on the N-terminal surface of the molecule, albeit the antiproteinase site lacks conserved residues (Župunski et al., 2003; Ranasinghe and McManus, 2013). These observations suggest that the same overall three-dimensional structure of snake venom KSPIs remains the same, though it has evolved to exert different biological functions (Cardle and Dufton, 1997; Župunski et al., 2003; Ranasinghe and McManus, 2013; Mukherjee et al., 2014a; Mukherjee and Mackessy, 2014).

Based on their functional properties, snake venom KSPIs are divided into trypsin and/or chymotrypsin inhibitors. Snake venom trypsin and/or chymotrypsin inhibitors are further categorised as either non-neurotoxic snake venom KSPIs or homologs of the Kunitz/BPTI group that are neurotoxic snake venoms (Cardle and Dufton, 1997; Župunski et al., 2003; Ranasinghe and McManus, 2013). The neurotoxic homologs, mostly isolated from the elapidae family of snakes, have lost their protease inhibitory function through adaptive evolution, and their biological activity is mostly confined to K^+ and Ca^{2+} channel blockers (Cardle and Dufton, 1997; Župunski et al., 2003; Ranasinghe and McManus, 2013). However, recent studies from our have demonstrated that KSPI complexes, isolated from the venom of Russell's viper, may also exert neurotoxicity in experimental mice (and possibly in human victims) by an unknown mechanism (Mukherjee and Mackessy, 2014; Mukherjee et al., 2016). Table 2 shows some of the salient features of protease-inhibitor KSPIs isolated from snake venom. A discussion of the pathophysiological significance and biomedical application of KSPI homologs with channel blocking (neurotoxic)