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Mini-Review

Venom neutralization by purified bioactive molecules: Synthetic peptide derivatives of the endogenous PLA_2 inhibitory protein PIP (a mini-review)

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ABSTRACT

Envenomation due to snakebite constitutes a significant public health problem in tropical and subtropical countries. Antivenom therapy is still the mainstay of treatment for snake envenomation, and yet despite recent research focused on the prospects of using antivenom adjuncts to aid in serotherapy, no new products have emerged so far for therapeutic use. Various methodologies including molecular biology, crystallography, functional and morphological approaches, etc., are employed in the search for such inhibitors with a view to generate molecules that can stop partially or completely the activities of toxic phospholipase A_2 (PLA_2) and snake venom metalloproteinase (SvMPs) enzymes at the molecular level. Herein, both natural and synthetic inhibitors derived from a variety of sources including medicinal plants, mammals, marine animals, fungi, bacteria, and from the venom and blood of snakes have been briefly reviewed. Attention has been focused on the snake serum-based phospholipase A_2 inhibitors (PLIs), particularly on the PLI derived from python snake serum (PIP), highlighting the potential of the natural product, PIP, or possible derivatives of it, as a complementary treatment to serotherapy against the inflammation and/or muscle-damaging activity of snake venoms. The data indicate a more efficient pathway for inhibition and blocking the activity of PLA_2s and matrix metalloproteinases (MMPs), thus representing a feasible complementary treatment for snakebites. Such information may be helpful for interfering on the biological processes that these molecules are involved in human inflammatory-related diseases, and also for the development of new drugs for treatment of snake envenomation.

1. Introduction

Snake envenoming is an important public health problem in many regions, particularly in tropical and subtropical countries. Envenomings by most viperid snakes and some elapids can cause local tissue necrosis, mostly due to the action of enzymes such as phospholipases A_2 and zinc-dependent metalloproteinases. Systemic effects due to envenomings include haemorrhage, coagulopathy, haemodynamic disturbances, and neurotoxicity caused by presynaptic and post-synaptic neurotoxins. The only remedy currently available for treatment of snake envenomations is antivenom. Although this form of therapy is in widespread use the world over and is currently the mainstay of treatment for snakebite, there are some drawbacks that must be taken into consideration: (1) antivenom use is associated with a high incidence of early and late adverse reactions; (2) specific antivenom containing broad spectrum of protective antibodies to treat envenomings by different species of snakes or same species from different

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geographical locations is not yet available; (3) antivenom effectiveness is limited to systemic envenomation only, being ineffective in most cases of local tissue damage; (4) most antivenoms are ineffective in the reversal of neurotoxicity induced by presynaptically acting phospholipases A_2s (e.g., β-bungarotoxin, crotoxin, taipoxin, mojave toxin, etc). Hence, it is essential to search for natural or synthetic bioactive molecules that could complement or substitute antivenom therapeutics.

2. Phospholipases A_2 (PLA_2s) and PLA_2-inhibitors (PLIs)

Toxicity of snake venom is often addressed to the activities of PLA_2s, one of the major components of snake venoms (Meier and White, 1995). Phospholipases A_2 (PLA_2s) are commonly found in snake venoms from Viperidae, Hydrophiidae and Elaphidae families. They exhibit a wide variety of pharmacological effects such as neurotoxicity, cardiotoxicity, myotoxicity, cytotoxicity, nephrotoxicity, anticoagulation, hypotension, and edema (Kini, 1997). Besides snake venoms, PLA_2s have also been found in mammalian systems. On the basis of cellular disposition and calcium dependence, PLA_2s are broadly divided into three classes, namely secretory PLA_2 ($sPLA_2$), cytosolic PLA_2 ($cPLA_2$) and calcium-independent PLA_2 ($iPLA_2$). Each class of PLA_2 is further subdivided into isoenzymes for which there are 10 for mammalian $sPLA_2$ (groups IB, IIA, IIC, IID, IIE, IIF, III, V, X, XIIA) (Six and Dennis, 2000), at least 3 for $cPLA_2$, and 2 for $iPLA_2$ (Gilroy et al., 2004). The PLA_2 superfamily includes 15 groups comprising five main types including the secreted $sPLA_2$, cytosolic $cPLA_2$, calcium-independent $iPLA_2$, the platelet activating factor (PAF) acetyl hydrolase/oxidized lipid lipoprotein associated (Lp) PLA_2, and the lysomal PLA_2s (Burke and Dennis, 2009). Interested readers should also consult other reviews for more details on PLA_2 nomenclature/classification (Six and Dennis, 2000; Schaloske and Dennis, 2006).

Because of their implication in a range of diseases including rheumatoid and osteoarthritis, asthma, acute pancreatitis, septic shock, etc., recent research has focused on the role of PLA_2-inhibitors (PLIs) as possible anti-inflammatory agents (Meyer et al., 2005), which may be useful as potential therapeutics for inflammation-related diseases or as antivenom-like bioactive molecules for snake venom neutralization. PLA_2 inhibitors specific to Group I and II PLA_2 isoforms are therapeutically important both against venom toxicity and as anti-inflammatory molecules.

3. PLIs from different natural sources

Because of the importance of PLIs for their therapeutic applications against inflammation and venom PLA_2 toxicity, a number of review articles on this topic are already available in the literature (see recent reviews by Marcussi et al., 2007; Sanchez and Rodriguez-Acosta, 2008). A valuable collection of latest information compiled in the recent special issue (Rangappa, 2007) comprehensively covers this particular area of research. In summary, PLIs have been identified from a variety of natural sources including medicinal plants, mammals, marine animals, fungi, bacteria, and from the venom and blood of snakes. A number of reports on plants from different geographical areas that are able to neutralize snake venoms are available (Abubakar et al., 2006; Soares et al., 2004, 2009), but only a few chemical compounds have been isolated and identified as active components (Soares et al., 2005; Narendra Sharath Chandra et al., 2007). The neutralization factors isolated from the serum of mammals like the opossum, mongoose, and hedgehog are all anti-hemorrhagic large multimeric proteins (Perales et al., 1994; Tomihara et al., 1987; Lizano et al., 2003). An antivenom substance isolated from opossum serum inhibits both the protease and PLA_2 activities (Rodriguez-Acosta et al., 1995). Although the marine-derived PLIs such as manoalide, scalaradial, petrosaspongiolide M-R, and their related compounds (Monti et al., 2007) have interesting anti-inflammatory properties, few marine compounds have entered clinical trials, requiring further developmental approaches to qualify as anti-inflammatory therapeutics (Alcaraz and Payá, 2006). PLIs have also been purified and characterized from sources like fungi and bacteria (Souza et al., 2008), and also from the primary sequences of group II PLA_2s from snake venoms and human, based on the native peptide inhibition method (Tseng et al., 1996; Church et al., 2001), but few have entered clinical developmental stage.

3.1. PLIs from snake blood

The PLIs that interact with PLA_2s and inhibit their enzymatic activity have been purified from the sera of venomous (Crotalinae, Viperidae, Elapidae) as well as non-venomous snakes (for details see recent reviews by Marcussi et al., 2007; Sanchez and Rodriguez-Acosta, 2008). Three types of PLIs have been classified: C-type lectin-like proteins (α-PLIs), molecules bearing leucine-rich repeats similar to human α2-glycoprotein (β-PLIs), and those with a three-finger configuration (γ-PLIs) analogous to proteins found in mammalian cell-surface Ly-6 antigens, elapid neurotoxins, and urokinase-type plasminogen activator receptor uPAR. All three types of PLIs have been identified in the sera of viperid snakes; whereas in the sera of elapid and hydrophid snakes, only PLIγ has been identified. The sera of non-venomous snakes, *Elaphe quadrivirgata* (Okumura et al., 2002) and *Elaphe climacophora* (Shirai et al., 2009) contain two homologous subunits, PLIγ-A and PLIγ-B, whereas *Python reticulatus* contains only a single PLIγ-A subunit (Thwin et al., 2000). *Crotalus durissus terrificus* CNF (dos Santos et al., 2005) has also been reported to be homomeric PLIγ like PIP. In addition, a single PLI member of the immunoglobulin supergene family has also been identified (Neves-Ferreira et al., 2009). For detailed information on the PLI proteins derived from both snake and mammalian blood, and on the classification, molecular and functional characterization of myotoxic PLIs, see reviews by Marcussi et al. (2007).

3.1.1. PLI from Python serum (PIP)

Our group has previously purified a PLI with potent antitoxic and anti-inflammatory activities from the serum of the non-venomous snake *P. reticulatus*. This PLI, initially