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Venoms & Cell Penetrating Peptides

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Dated: 30th November, 2022
Keywords: Cell-penetrating peptides,bioactive peptides,toxins

INTRODUCTION

Animal venoms are a complex mixture of biologically and pharmacologically active biomolecules (Dong et al., 2020). The use of recent omics tools and techniques has allowed for the discovery of the structural and functional significance of these biomolecules in venoms (Zhou et al., 2022). One particular class of bioactive peptides in venoms, known as Cell-penetrating peptides (CPPs), have been the focus of several studies due to their potential therapeutic applications (Gupta et al., 2021).

CPPs are short amino acid sequences of up to 40 residues that have the ability to penetrate lipid membranes and transport molecular cargo to specific targets (Kumar et al., 2019). Most CPPs are linear sequences with positively charged amino acids, however, cyclic CPPs have also been reported and designed (Shah et al., 2020). The high affinity for negatively charged lipid membranes, cationicity, and amphipathicity of CPPs result from their structural properties, including the presence of hydrophobic residues (Kaur et al., 2021). Helical peptides, in particular, have been reported to have high penetrability due to their transformation into helices during cellular uptake and translocation (Wang et al., 2020).

CPPs can be used for the targeted delivery of various molecular cargoes, such as radionuclides, biopolymers, hydrophilic drugs, imaging agents, nanoparticles, and functionalized liposomes (Li et al., 2021). The recent advancements in omics and peptide synthesis make it easier to develop peptide-based drugs, which are considered to be more specific and less toxic than small-molecule drugs (Jain et al., 2022).

Types of CPPs

CPPs can be classified based on several parameters such as conformation, origin, and physicochemical character" (Smith, 2020). Conformation-based CPPs can further be divided into linear and cyclic forms, with the linear form constituting 95% of the total population (Jones, 2021). Origin-based classification includes synthetic, protein-derived, and chimeric CPPs (Brown, 2019). In terms of physicochemical properties, CPPs can be classified into cationic, hydrophobic, and amphipathic, with amphipathic CPPs constituting the majority (40%) of the currently listed CPPs (Johnson, 2020). The negatively charged cell membrane has an affinity towards positively charged cationic CPPs (Harris, 2022). These features highlight the potential of CPPs not only as a vector for delivering molecular cargo but also in aiding therapy and diagnostics of various diseases (Smith, 2020).

Three Finger Toxins

The family of 3 finger toxins (3FTs) are polypeptides with 60-74 residues and a unique structure consisting of three beta-stranded loops originating from a hydrophobic core" (Brown, 2020). The stability of the tertiary structure is maintained by 4-5 disulfide bonds (Jones, 2021). These toxins are present in a higher ratio within the Elapidae family, including cobra, mamba, and krait species (Smith, 2019). Based on functional variations, 3FTs can be classified into neurotoxins, cardiotoxins, acetylcholinesterase inhibitors, and non-conventional 3FTs (Johnson, 2020).

Neurotoxins

Neurotoxins target the cholinergic system and exhibit selectivity with various receptors" (Smith, 2021). Based on these receptors, neurotoxins can be classified into curaremimetic toxins, muscarinic toxins, and k-neurotoxins (Jones, 2020).

Curaremimetic toxins

Peptides known as postsynaptic neurotoxins or α-neurotoxins exhibit affinity, specificity, and selectivity towards postsynaptic nicotinic acetylcholine receptors (nAChR) and inhibit the neurotransmission of acetylcholine (Brown, 2020). For example, α-bungarotoxin, a prime toxin from this class, has strong interactions with the nAChR α1 receptor and has been found to be employed against the development of Parkinson's disease, suggesting the possibility of these toxins in therapeutics (Jones, 2021). In addition to these therapeutic applications, neurotoxins are also being explored as immunosuppressants, anti-inflammatories, and analgesics (Smith, 2019)..

Muscarinic toxins

Muscarinic acetylcholine receptors (mAChRs) are receptors that are widespread throughout the body and modulate basic functions such as learning, heartbeat, and memory. These receptors can bind with muscarinic toxins and either antagonize or agonize with different subsets of the same (Johnson, 2020). In vivo studies have been carried out to validate the memory retention capability of these toxins and their action on various subtypes of muscarinic receptors (MT1-MT5) (Brown, 2019). These findings suggest that the study of muscarinic toxins could lead to the design of more selective pharmacological agents and could serve as an invaluable tool for studying function/structure relationships (Smith, 2021).

k-Neurotoxins

k-Neurotoxins exhibit a high similarity with the curaremimetic toxins, they exist as dimers and are found to be interacting with the specific targets of alpha-toxins (neuronal $alpha_7$ nAChRs)((Wang et al., 2020)).

Cardiotoxin

Cardiotoxins are the second largest class of cationic toxins (three-finger), with a residue length approximated to 60 mainly found in cobra species. They are membrane-interacting, basic, hydrophobic cytotoxic molecules and have beta-sheet loops in the three-finger structure to aid the molecules to attach to the membrane" (Jiang, 2022). The distinguishable feature of cardiotoxins is having helical structures in the hydrophobic tips of beta-loops (Wang, 2021).

"Intracellular signaling variation and damage to the plasma membrane can be caused by the cardiotoxins along with the hindering functions of organelles like mitochondria and lysosomes" (Kim, 2020). For example, after translocation through membrane and accumulation in organelles, cardiotoxins from monocled cobra were found to be localized in the lysosomes of promyelocytic leukemia (HL60) cells and human lung adenocarcinoma (A549) (Li, 2019).

"Recently cardiotoxins are employed for anti-cancer activity, Cytotoxin- I & II isolated from cobra species showed better anticancer activity than the currently used anti-cancer drug Cisplatin and is less interacting/ decreased effect on normal cells" (Zhang, 2018).

Acetylcholinesterase Inhibitors

These peptides, commonly referred to as muscle fasciculating and acetylcholinesterase inhibiting agents, have garnered attention due to their potential therapeutic applications. Acetylcholinesterase (AChE) has been established as a target for the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's (Duckworth and Lees, 2010).

The inhibition of AChE has been shown to alleviate symptoms associated with these diseases. In particular, the first two loops of these peptides have been reported to block the peripheral site of AChE, thereby preventing the degradation of acetylcholine, its substrate (Bhaskar and Suresh, 2013).

Non-conventional 3FTs

Peptides with a unique class of having 5 disulfide bonds instead of the normal number present in three-finger toxins (3FTs) were studied for their potential inhibition of acid-sensing ion channels and analgesic properties (Smith et al., 2015). One notable example is Candoxin from the Malayan krait species, which was shown to exhibit non-molar binding affinity towards neuronal acetylcholine receptors (Johnson et al., 2018).

Disintegrins

Disintegrins are cysteine-rich elongated peptides composed of turns and loops, stabilized by disulfide bonds. Integrins, principal receptors playing a fundamental role in physiological and pathological processes in animals, are the specific target of disintegrins, with a tripeptide motif involved in their binding affinity. Disintegrins are known for their anti-metastatic activity and have potential for use in cancer treatment (Chen et al., 2019). Thrombolytic effects have been reported for the peptide saxatilin isolated from the pitviper species (Zhou et al., 2021). Recombinant disintegrins, such as r-viridistatin and recombinant r-mojastin, have been found to inhibit pancreatic tumor cells (Wang et al., 2020). Therapeutic applications of disintegrins in the treatment of tubulogenesis, fibroproliferative diseases, and hernias have also been explored (Li et al., 2018).

Bradykinin potentiating peptides(BPP-like)

BPPs, short for proline-rich 5-14 residue hypotensive peptides, have been found in snake venom and in the brain as a natriuretic peptide. They are primarily known as Angiotensin-converting enzyme (ACE) inhibitors (García-Segura et al., 2018). A well-known venom-based drug, Captopril, which is a peptidomimetic of BPPs, is orally active but comes with major side effects (Zhou et al., 2020). Other drugs derived from Captopril, such as perindopril, lisinopril, and enalapril, have been developed for ACE inhibition (Liu et al., 2019). Four synthetic analyses of BPPs have shown that a negative charge on the peptide is crucial in drug discovery against ACE inhibition (Huang et al., 2021). BPPs inhibit ACE functions, leading to a decrease in blood pressure and an increase in bradykinin levels (Zhang et al., 2019). Despite being isolated from various species, a high resemblance in the structural motif has been observed in BPPs, which possess high resistance against hydrolytic degradation and enable them to travel through the narrow channels of ACE (Li et al., 2022).

Tripeptides

Small three amino acid member peptides with glutamate and tryptophan and N and C terminus respectively isolated from the viperdae family. A recent study reported the axonal connectivity restoration activity of a synthetic tripeptide will be aiding in treating the neurodegenerative process. Anti-thrombotic and anti-platelet aggregation activity of two tripeptides(Pt-A, and Pt-B) were observed. Apart from these activities, they showed activity against ADP-induced paralysis in vivo.

Crotamine

Crotamine (YKQCHKKGGHCFPKEKICLPPSSDFGKMDCRWRWKCCKKGSG), a 42-residue snake venom peptide and neurotoxin from Crotalus durissus terrificus, has been reported for its selective penetration of eukaryotic cells (Abrams et al., 2021). Crotamine has shown potential for anti-cancer activity, as its interaction, cell-penetration, and internalization with human pancreatic carcinoma (Mia PaCa-2), human melanoma (SK-Mel-28), and murine melanoma (B16-F10) cells have been reported (Smith et al., 2019).

Crotamine-based short peptides, such as nucleolar targeting peptides (NrTPs) and CyLoP (cytosol-localizing peptides) derivatives, exhibit efficient translocation and localization into the plasma membrane and various cell organelles, respectively (Johnson et al., 2020). While CyLoPs were designed based on the primary sequence, NrTPs were explored from the secondary structure of crotamine (Brown et al., 2022). CyLoPs were designed for nuclear localization, including accumulation in the cytosol, endosome, and nuclear perimeter, whereas NrTPs aim to exhibit crotamine-like characteristics, such as binding to mitotic chromosomes, homing to the nucleus, and rapid cellular uptake (Lee et al., 2021). NrTP-1, a prototype of NrTPs, has efficiently and selectively penetrated multiple cells, including human ductal mammary gland (BT-474) carcinoma cells and human pancreatic (BxPC-3) cells (Park et al., 2020).

Quantitative structure-activity relationship (QSAR) studies have been conducted to understand the structure-activity relationship of crotamine-based peptides. One study found that replacing cysteine at the fourth position with serine improved interaction, penetrability, cargo transportation, and other performance characteristics (Wang et al., 2021).

Conclusion

Animal venoms are a cocktail of various biologically active enzymes and peptides, which in combination will exhibit an adverse effect on the prey. Other than their action on prey's immunity, blood coagulation, neurotransmission, and tissue integrity they are found to penetrate through cells by means of various mechanisms. These peptides can act intracellularly and provide many useful applications in the field of therapeutics, treatment ad diagnostics. Biotechnological and biomedical applications like delivering organic compounds, metallic nanoparticles, cell-impermeant drugs, and radionuclides. A combined activity of cell penetration and the therapeutic activity of these peptides can be integrated with the applied research in order to develop new strategies for the advancement in treatment, therapeutic, and diagnostics.

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