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ANTIBACTERIAL ACTIVITY OF AN ACIDIC PHOSPHOLIPASE A2(,NN-XLB-PLA2) FROM THE VENOM OF *NAJA NAJA* (INDIAN COBRA)

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ABSTRACT

Bacterial resistance against common antibiotics has become a critical condition against human health and has serious side effects. To overcome these problems, new methods to develop new molecules have received special attention in recent years. This review studies the antibacterial activity of an acidic protein NN-Xib-PLA2 (Naja naja venom phospholipase A2 fraction— Xib) of Naja naja venom experimented; the result showed great antibacterial activity against gram+ve bacteria like Bacillus subtilis and gram-ve bacteria like Klebsiella pneumoniae, Escherichia coli. Minimum inhibitory concentration value from 1720ug/ml. The result showed a strong correlation between PLA2 activities and hemolytic and antibacterial activity. The result also showed that in the presence of p-bromophenol bromide, there is a significant inhibition of enzymatic activity with bactericidal effects. These studies show the different paths to studying molecular mechanisms of antibacterial properties of NNXib PLA2, which will help to develop this protein into a possible therapeutic lead molecule to treat bacterial infection. Snake venoms are complex mixtures; mainly it has proteins that have enzymatic activities, inorganic cations, calcium, potassium, magnesium, zinc, nickel, cobalt, iron and manganese. Zinc is necessary for anti-cholinesterase activity; Calcium is required for the activation of enzymes such as phospholipase. Some snake venoms also contain carbohydrates, lipids, biogenic amines, and free amino acids. Proteins found in snake venom include toxins, neurotoxins, non-toxic proteins, and many enzymes, especially hydrolytic ones. Enzymes are inherently proteins, including digestive hydrolases, L-amino acid oxidase, phospholipases, thrombin-like procoagulants, and kallikrein-like serine proteases and metalloproteinases (hemorrhagins) that damage vascular endothelium. Phosphodiesterase enzymes disrupt the prey's cardiac system, primarily to lower blood pressure. Phospholipase A2 causes hemolysis by lysing the phospholipid cell membrane red blood cells. Amino acid oxidases and proteases are used for digestion. Amino acid oxidase also triggers some other enzymes and is responsible for the yellow color of the venom. Hyaluronidase enzymes increase tissue permeability to accelerate uptake of other enzymes into tissueSnake venom is being studied for the treatment of many diseases such as cancer, high blood pressure, and thrombosis. Venoms from rattlesnakes and other crotalids produce changes in blood vessel resistance, changes in blood cells and clotting, and changes in heart and lung dynamics. The individual effects of all toxins integrate into fatal dysfunctions of almost every organ system. Such a toxin mimetic can help to pharmaceutically influence a specific bodily function for the benefit of human health. Such snake toxin-derived mimetics are in clinical use, trials or consideration for further pharmaceutical use, particularly in the areas of hemostasis, thrombosis, coagulation and metastasis. Snake venom has great potential as a medicine because of all the compounds it contains and their specific effects. Two analgesics come from cobra venom; Cobroxin, like morphine, is used to block nerve transmission and nyloxine reduces severe arthritis pain. The Arvin compound from the Malayan pit viper is a potent anticoagulant. Venom components allow researchers to develop novel drugs to treat many diseases, such as neural epilepsy, multiple sclerosis, myasthenia gravis, Parkinson's disease and poliomyelitis, musculoskeletal disorders. The combination of snake venoms (SVs) could synergistically enhance the antiproliferative effects at low doses on liver cancer cells (HepG2). In such researches, gene expression for apoptotic, inflammatory, antioxidant and cell cycle regulators has been determined. Various compounds from venomous animals such as spiders, scorpions, snakes, caterpillars, centipedes, wasps, bees, toads, ants, and frogs have widely shown biotechnological and pharmacological applications against many diseases including cancer. Snake venoms have been reported to have cytotoxic effects against tumor cells. This potency is based on the inhibition of cell proliferation and the promotion of cell death by activating the apoptotic mechanisms.

that control the cell cycle, and treated initiating damage in cell membranes. The complex mixtures of snake venom, L-amino acid oxidase (LAAO) act as anticancer therapeutic activity and by inducing oxidative stress in cancer cells. L-amino acid oxidase (LAAO) has been reported to have potent antitumor activity against various cancer cell lines including. LAAO can selectively bind to the cancer cell surface at specific phospholipid compositions to release the hydrogen peroxide. LAAO mediates its cytotoxicity to the cell surface and produces H2O2. In addition, studies confirm this safer effect on animal models. In terms of cytotoxicity, combined administration of LAAO with SOR reduced cell death on normal liver cells THLE-2 compared to single administration. On the other hand, administration of LAAO and SV alone or in combination with SOR significantly induced cell death and apoptosis in HepG2 cells compared to untreated control cells. Furthermore, showed that LAAO isolated from Ophiophagus hannah venom selectively kills cancer cells via the apoptotic pathway by regulating caspase 3,7 activity, but is non-toxic to normal cells. One of the consequences of excessive damage caused by the reactive oxygen species (ROS) are changes in mitochondrial membrane permeability causing Ca+2 overload leading to cytochrome c release and apoptotic death.

KEYWORDS: Bactericidal effect, snake venom, antimicrobial resistance, L amino acid oxidase.

INTRODUCTION

Antimicrobial resistance is one of the major public health risks worldwide, including in developed countries of Europe. According to the World Health Organization (WHO), infections due to multidrug-resistant (MDR) strains are among the most common causes of death worldwide. In particular, there is increasing evidence of the e mergence and spread of antibiotic-resistant bacteria. In addition, previous studies have shown that inappropriate use of antibiotics such as vancom ycin can lead to vancomycin- resistant strains such as vancomycin intermediate (VISA) and enterococci. Similarly, various bacteria such as Pseudomonas, Klebsiella, Enterobacter, Acinetobacter and Salmonella are re sistant to multiple antibiotics. Thus, laboratory- and clinical-based research geared toward discovering new and potent bactericidal candidates with unique mechanisms of action that could overcome antimicrobial resistance is warranted. Studies on crude snake venoms and/or their fractions often result in potential therapeutic mol ecules against bacteria and other parasites. Snake venoms are composed of a spectrum of protein-based constituents. These components could be categorized into four broad groups , namely: (1) The dominant group, which consists of the three-finger toxins (3FTx), phospholipases A2 (PLA2), snake venom metalloproteases (SVMP), and snake venom serine proteases (SVSP), (2) The second group consists of a small number of proteins, which includes Kunitz peptides (KUN), cysteine-rich secretory proteins (CRiSP), L-amino acid oxidases (LAAO), C-type lectins (CTL), disintegrins (DIS), and natriuretic peptides (NP), (3) The third group contains the rarely observed snake venom proteins, including venom nerve growth factor (VNGF), vascular endothelial growth factor (VEGF), acetyl cholinesterases, hyaluronidases, 50-nucleotidases, phosphor diesterases (PDE), and snake venom metalloprotease inhibitors, and (4) finally, the fourth group includes cobra venom factors (CVF), galactose-binding proteins, aminopeptidases, and waprins. However, the proteins mentioned above may only be readily available in some venomous snakes. Cobra snakes, for example, have Group I PLA2 and 3FTx, while mamba is known to be composed primarily of Kunitz peptides (also known as dendrotoxins). Viperid consists of PLA2 and proteases as the most abundant protein group containing different amounts of serine proteases and metalloproteases. In this study, we present a thorough literature-based review of the bactericidal activity of the snake venom PLA2, along with an analysis of the reported minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) against bacteria. The listed study strains highlight the potential of snake venom fraction as a promising candidate against some species of antimicrobial-resistant bacteria.

STRUCTURE OF PHOSPHOLIPASE: PLA2 represents a class of thermostable Ca2+-dependent enzymes that catalyze the hydrolysis of the 2-acyl bond of 3-n-phosphoglycerides. PLA2 is involved in lipid metabolism and represents important probes for structure-function relationships in biological membranes.[1,2] Secretory PLA2 of groups I and II (sPLA2) are small (10–14 kDa) compact proteins measuring approximately 22 x 30 x 42 Å. Fourteen conserved cysteines of sPLA2 form seven disulfide bonds that stabilize the tertiary structure. These proteins share a homologous core and an invariant tertiary structure.[27, 28] These include three α-helices (residues 1–12, 37–54, and 90–109) with a characteristic main loop (residues 24–30). Amino acid side chains arising from the homologous core coordinate primary Ca2+, define the substrate binding pocket, and mediate the electronic events of catalysis. Two of the three conserved α-helices and the Ca2+ binding loop are also present in bee venom group III sPLA2 (residues 25–37, 61–74, and 8–12).[5]

The “missing” helix of groups I and II is replaced in the bee venom sPLA2 by a single helix that arises at a non-homologous position. Comparisons of the crystal structures of the sPLA2 isoenzymes from Micropechis ikaheka (MiPLA2, MiPLA3 and MiPLA4 show different pharmacological effects) show that the C-terminus (residues 110–124) is more variable. The C-termini of the medium (MiPLA4) and high potency (MiPLA2) isoenzymes group together and form a highly exposed surface. An analogy of the sequence to the stronger MiPLA2 sequence, performed using the BLAST database search engine, results in high similarity to coagulation factor A and cadherin 11 of Staphylococcus

aureus.

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FUNCTIONAL CHARACTERISTICS OF THE PLA2 FROM NAJA SPP. VENOM

The envenomation process is initiated by PLA2s from animal venoms, which also cause bleeding, edema development, platelet aggregation, hemolysis, myotoxicity, cardiotoxicity, and neurotoxicity.[9] The PLA2s' catalytic function, which acts on the phospholipids that comprise cell membranes and causes their lysis to release arachidonic acid, is partly responsible for the pharmacology and toxicology of PLA2s. This acid affects immunological response and inflammatory processes and is a precursor to bioactive lipids (prostaglandins, leukotrienes, and thromboxanes).[10] Furthermore, PLA2 derived from the venom of Naja mossambica exhibited antiviral efficacy against members of the Flaviviridae family, including the Japanese encephalitis virus (JEV), dengue virus (DENV), and hepatitis C virus (HCV).[12] For these enzymes to establish their activity, their structure is essential. To keep the molecule's contact with the solvent intact, hydrophilic amino acids are found on its surface. However, the hydrophobic amino acids are oriented toward the protein nucleus, where they produce a hydrophobic channel that directs the lipid substrate to the enzyme's catalytic site.[11] The amino acid residue Asp 49—or Cys 49 in Naja sagittifera—is necessary for the binding of the calcium ion, which the PLA2s require as a cofactor. The overlapping molecular structures, this domain is substantially conserved in PLA2s from Naja spp. venoms. The overlapping molecular structures of PLA2s from Naja spp. venoms indicate a high degree of conservation of this domain. The calcium ion found in PLA2s is coupled to two molecules of water 55 and the oxygen of the carbonyl group of Asp 49 in the N. naja naja snake toxin. Other divalent ions, including barium or cadmium, can substitute for calcium, but doing so reduces PLA2 activity.[13] Changes in the pharmacological qualities are caused by variations in other amino acid residues, as Condrea et al. have shown of enzymes and at the enzyme catalytic site. Modification of the amino acid lysine (Lys) in phospholipases of Naja naja atra and Naja nigricolis was shown to reduce pharmacological efficacy. However, the activity of the poison did not show a significant decrease in the catalytic activity. However, modification of the arginine residue (Arg) (alkalinization) affects both the catalytic activity of PLA2 and its catalytic activity.

pharmacological properties, suggesting that modifications to amino acids that are not essential for catalytic activity would also modulate the catalytic activity of the enzyme. Another binding site of ultimate importance for enzymatic activity is the N-terminus. It is an activation region of PLA2 and functions to help the enzyme to assume more stable conformation for the degradation of aggregate substrates, allowing it to find more orientations. productive for the reaction on the membrane surface. Therefore, maintaining the integrity of the N-terminus is crucial to preserve the active conformation of the PLA2 enzyme. The N-terminal end shows a specific affinity for lipid-water interfaces, which may be due to the fact that the region of the molecule has hydrophilic and hydrophobic properties.The Ca 2+ ion acts at the N-terminus and causes it to rotate on its own axis, exposing the amino acid residues so they can bind to the enzyme substrate. Changes to the N-terminus affect the structure of the catalytic site and subsequently alter the structure of the enzyme.[14]

Alkalization of His 48 and its modification by p-bromophenacyl bromide (BPB) or replacement of strontium ions with calcium ions reduces the catalytic activity of the enzyme because the enzyme cannot bind efficiently to the substrate. It is important to note that the neurotoxic effect developed by these enzymes is related not only to PLA2, but also to synergism with neurotoxic toxins such as crotoxin, taipoxin and β-bungarotoxin.[13] A similar study by Rudrammaji et al has shown that the effect of PLA2-induced platelet aggregation may be related to the decrease in membrane permeability, allowing direct modification of platelets through the catalytic action of these enzymes. They also showed that modifications in His 48 caused a decrease in catalytic function and inhibition of platelet aggregation induced by ADP, collagen and adrenaline. PLA2 named Nigexine, isolated from Naja nigricollis, showed in its sequencing 118 amino acid residues and 14 hemicystine residues and was homologous to other basic A2 phospholipases from mammals and reptiles. The enzyme showed cytotoxic activity but was independent of the presence of divalent ions and therefore was not related to

esterase activity.

Nigexin showed higher catalytic activity and lower lethal dose.In contrast to treatment with cyanogen bromide, which resulted in almost complete loss of enzymatic activity and no cytotoxicity was detected, modification with p-bromophenacil bromide inhibited all enzymatic activity while retaining some cytotoxicity.[15]

The potential to hydrolyze phosphatidylcholine was observed in nigexin, but was absent in notexin, bungarotoxin, bee basic PLA2, and porcine pancreatic PLA2, suggesting the involvement of three regions (residues 1–22, 55–96, and 102–125) in inducing this effect.[16] PLA2 from Naja spp. The poisons have also been described as bactericidal. The acidic protein NN-XI-a-PLA2 (Phospholipase A2 Fraction-XI-a of Naja naja venom) shows bactericidal activity on gram-positive and gram-negative strains.Minimum inhibitory concentration (MIC) values in the range of 17 to 20 µg/ml demonstrate antibacterial activity comparable to that of commonly used antibiotics. After modification of PLA2 with p-bromomphenacil bromide (BPB), there was a significant decrease in enzymatic and antimicrobial activities, suggesting the existence of a correlation between them as they are related to the destabilization of the lipid bilayer in the membrane. and thus increases the susceptibility of bacterial cells to osmotic lysis.[17]

Stefansson et al. described a phospholipase fraction isolated from Naja nigricollis that is capable of inhibiting the prothrombinase complex by a mechanism independent of enzymatic activity. The result showed a 97% reduction in enzymatic activity after alkylation of the histidine residue and a prothrombinase inhibitory potential of 60%.This result differed from other phospholipase fractions obtained from the same venom, which had abolished activity on prothrombinase.[18]

METHODS

Analyzing 150 reports retrieved from diverse sources, we selected a set of 26 studies for our investigation. The dual approach of database searches and manual identification yielded a comprehensive pool, with 20 studies meet the eligibility criteria. Exclusion criteria at various stages refined the selection, and our thorough examination is detailed in Figure 1. Table 1 captures distinctive features of the chosen studies, while Table 2 presents essential data on MIC, MBC, and PLA2 sequences. This research journey is visualized in the flowchart, showcasing our meticulous search and screening procedures.

Table:1

Study	Snake Specie(s)	PLA2s	Bacterial Specie(s)	Activity on Bacterial Strains
Sudarshan et al	Najanaja	PLA2 (NN-XIb-PLA2)	S. aureus, Bacillus subtilis, E. coli	Inhibited the growth of all isolates, but more active on S. aureus and B. subtilis.
Nunes et al.	Bothrops Erythromelas	BE-I- PLA2	Acinetobacter baumanniii,Staphylococcus aureus.	Showed bactericidal activity against S.aureus and antibiofilm activity against A. baumanniii.
Alves et al.	Crotalusdurissusterrificus	Crotoxin PLA2-Crotoxin B	Ralstonia solanacearum	PLA2-CB showed 52% growth inhibition.
Vargas et al.	Porthidiumnasutum	PnPLA2	E. coli (ATCC 25922), S. aureus (ATCC 25923)	Showed bactericidal activity against S. aureus in a dosedependent manner but not on E. coli.
Samy et al.	Echiscarinatus	PLA2-EcTX- I	Bulkholderia pseudomallei (KHW and TES), Enterobacter aerogenes, E. coli	Strong bactericidal activity was observed in B. pseudomallei (KHW) and E. aerogenes. It showed only moderate effect on other bacteria
Sudarshan et al.	Daboia russelliipulchella	PLA2 fraction V (VRV-PL-V)	S. aureus. B. sub, E. coli, V. cholerae, K. pneumoniae, S. paratyphi	Exhibitedbactericidalactivity against S.aureus and B. subtilis more than On E.coli,V. cholerae, K.pneumoniae, S. paratyphi
Torres et al.	B. marajeonsis	Bmarajeonsis	P. aeruginosa, S. aureus	Could not promote any inhibitory activity
Samy et al	C. adamanteus	PLA2-CaTx- II	S. aureus, B. pseudomallei (KHW), B. pseudomallei (TES), E. coli, K. pneumoniae	Resulted in bactericidal effect by forming pores and damaging the cellwall membrane of the bacterial isolates.
Jia et al.	Agkistrodon piscivorusleucostoma	PLA2	B. subtilis, S. aureus, E. coli, V. cholera	A. pleucostoma PLA2 proteins namely AplAsp49 and AplLys49 did not show any bactericidal activity against any of the bacterial isolates.

Samy et al.	A. halys	PLA2-AgkTx-II	S. aureus, P. vulgaris, P. mirabilis, B. pseudomallei, E. coli	Caused potent bactericidal activity against S. aureus, P. vulgaris, P. mirabilis, and B. pseudomallei with rapid killing effect on S.aureus, P. vulgaris, B. pseudomallei in a dose dependent pattern. It was suggested that the activity was through membrane permeability and damage.
Samy et al.	C. durissuterrificus, Vipera ammodytes ammodytes, C. scutulatusscutulatus, Bungarusmulticinctus, Oxyuranus scutellatusscutellatus, Pseudechis australis, D. russelli	C.d.t- CA C.d.t-CB V.a.a-a C.s.sm B.m- β-b O.s.s-t P.a-m D.r-d	B. pseudomallei (KHW), B. pseudomallei (TES)	Presented bactericidal activity which was incriminated to be due to activity of cytotoxin and the PLA2.
Samel et al.	Viperalebetina, V. berusberus, N. oxiana,	NNOPLA2, VLPLA2, VBBPLA2	B. subtilis, E. coli, Vibrio fishera, S. aureus.	Only VBBPLA2 from V. berusberus completely inhibited the growth of B. subtilis. Moreover, the effect of VBBPLA2 was reported to be due to other properties of the protein rather than catalytic activity. To S.aureus, NNOPLA2 (from Najanaja)inhibited its growth and resulted in just a slight inhibition of the growth of B.subtilis. However, none of the three svPLA2s showed inhibitory effect on E. coli even at the highest concentration tried
Roberto et al.	B. jararacussu	BthA- IPLA2	E. coli (ATCC 29648) S. aureus (ATCC 25923)	Presented bactericidal activityagainst both bacteria.
Xu et al.	Bungarusfasciatus	BPFA-PLA2	E. coli, S. aureus	Showed activity against both bacteria.
Corrêa et al.	B. neuwiediurutu	BnuTX-I PLA2	E. coli (ATCC 25922), S. aureus (ATCC 29213), K. pneumoniae(ATCC13883) P.aeruginosa(ATCC27853)	Showed bactericidal activity against both Gram- positive and Gram-negative isolates, with greatest inhibitory effect on P. aeruginosa.
Denegri et al.	B. alternatus	Ba SpII RP4-PLA2	S. aureus (ATCC 25923), E. coli (ATCC 25922)	Showed no bactericidal activity against the two bacteria.

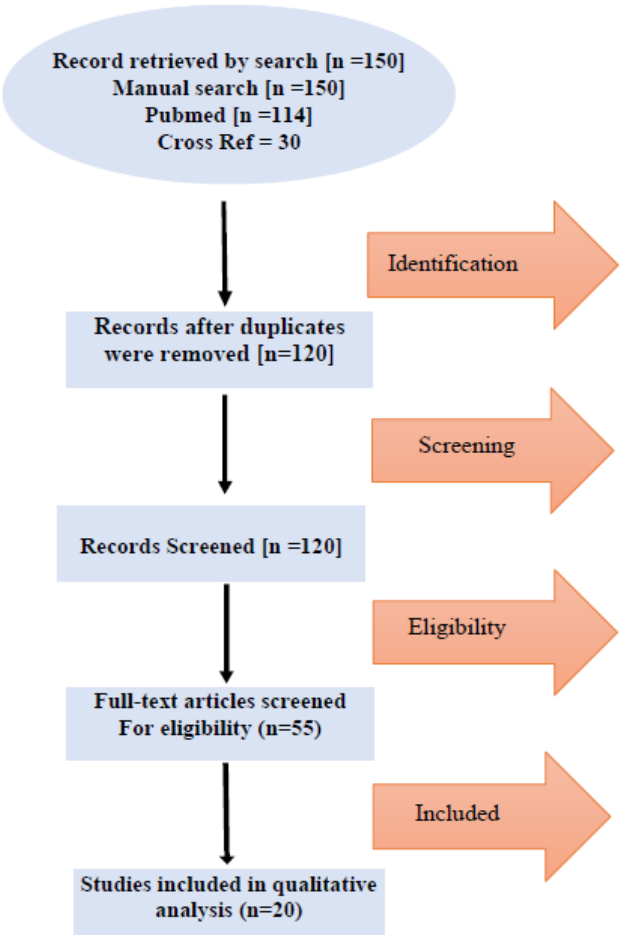

Fig 1: PRISMA diagram for the study search and selection processes.

Table 2.

Author	Minimum Inhibitory Concentration(s) (MIC)	Minimum Bactericidal Concentration (MBC)
Nunes et al.	NA	NA
Sudarshan et al	26.1 ± 3 µg/mL 21.3 ± 2 µg/mL 23.3 ± 3 µg/mL 25.1 ± 1 µg/mL 19.3 ± 3 µg/mL 21.4 ± 2 µg/mL	NA
Alves et al.	NA	NA
Vargas et al.	32 µg/ml DPIA	32 µg /mL DPBA
Samy et al.	DPIA 120 µg/mL 60 µg/mL 60 µg/mL 30 µg/mL 60 µg/mL 15 µg/mL 60 µg/mL	DPBA 18 µg/mL 26 µg/mL 25 µg/mL 2 µg/mL 9 µg/mL 1 µg/mL 22 µg/mL
Sudarshan et al.	13 ± 2 µg/mL 12 ±3 µg/mL 15 ±1 µg/mL 12 ±2 µg/mL 14 ± 3 µg/mL 12 ± 3 µg/mL 13 ± 1 µg/mL	NA

Torres et al.	DPIA	DPBA
Samy et al.	7.8 µg/mL 7.8 µg/mL 15.6 µg/mL 62.5 µg/mL 250 µg/mL 31.25 µg/mL 62.5 µg/mL 31.25 µg/mL 125 µg/mL	7.8–15.6 µg/mL 7.8–15.6µg/mL 7.8–15.6 µg/mL NA NA NA NA NA NA
Jia et al.	DPIA	DPBA
Samy et al.	10.63 µM -21.25 µM 21.25 µM 85 µM 42.5-85 µM 21.25 µM	NA
	(KHW)—10.63 µM 85 µM	
Samel et al.	NA	NA
Roberto et al	NA	NA
Xu et al	NA	NA
Corrêa et al.	NA	NA
Denegri et al.	NA	NA
Abid et al.	NA	NA
Barbosa et al.	NA	NA
Shebl et al.	B.a—NA P.a—625 µg/mL N.g—NA N.n.n—625 µg/mL V.l—NA E.c—NA C.c—1250 µg/mL B.a—312.5 µg/mL P.a—156 µg/mL N.g—312.5 µg/mL N.n.n—156 µg/mL V.l—625 µg/mL E.c—625 µg/mL C.c—625 µg/mL B.a—NA P.a—312.5 µg/mL N.g—625 µg/mL N.n.n—312.5 µg/mL V.l—625 µg/mL E.c—1250 µg/mL C.c—1250 µg/mL B.a—NA P.a—NA N.g—1250 µg/mL N.n.n—1250 µg/mL V.l—NA E.c—NA C.c—NA	NA
Almeida et al.	NA	NA
Santamaría et al.	NA	NA

SNAKE VENOM AS ANTIBACTERIAL AGENT

Although many articles have described the toxicity, structure/function, and mode of action of svPLA2 toxins, very few have reported their antimicrobial activity. This article uniquely summarizes the entire antimicrobial nature of svPLA2. Microorganisms naturally generate resistance to antibiotics through various mechanisms (Fig. 3), which are genetically determined.[19] The rapidly

growing prevalence of bacterial strains resistant to conventional antibiotics has led to an intensive search for new therapeutics, including several antimicrobial peptides of animal origin.[20] The discovery of new classes of antimicrobial peptides occurring in bacteria, lower eukaryotes and plants with wider distribution provides a new therapeutic strategy against microorganisms. Recent studies show that several cationic and noncationic peptides expressed in many species of vertebrates, invertebrates and bacteria act synergistically to enhance the immune response. Several snake venoms, including those of Daboia russelli russelli, Crotalus adamanteus, Naja sumatrana, Pseudechis guttata, Agkistrodon halys, Acanthophis praelongus, Pseudechis australis and D. russelli siamensis, have antibacterial activity against various bacteria.[21]

The poisons of Daboia russelli russelli and Pseudechis australis show strong inhibition against Staphylococcus aureus, while other poisons show only moderate activity against one or more bacteria. The susceptibility of several bacterial species to Viperidae toxins is S. aureus > Proteus mirabilis > Proteus vulgaris > Enterobacter aerogenes > Pseudomonas aeruginosa and Escherichia coli. Furthermore, natural venom peptides and svPLA2 possess potent antimicrobial activity against Burkholderia pseudomallei.[21,22] The bactericidal effect of the venom of Bothrops alternatus is greater against E.coli and S. aureus versus P. aeruginosa and E. faecalis.[23] Poisons from Bothrops moojeni and B.Jararacussu also inhibits the growth of Streptococcus mutans.[24] Another study shows that the entire venom as well as certain venom enzymes of Bothrops marajoensis inhibit the growth of several microorganisms, including S. aureus, P. aeruginosa, Candida albicans and Leishmania sp.[25] Crotoxin B and daboiatoxin purified from PLA2 have strong antibacterial activity against gram-negative B bacteria.pseudomallei (TES strain). Interestingly, a metalloproteinase from A. halys (AHM) was found to have antibacterial properties and was highly active against S. aureus, P. vulgaris, P.mirabilis and multi-resistant B. pseudomallei (strain KHW). AHM variants with high bacteriostatic activity (minimum inhibitory concentration or MIC = 2–60 µM) also tended to be less cytotoxic to human monocytic U-937 cells up to concentrations of 1 mM.[26] Crotamine inhibits multiple strains of E.coli with MIC values of 25–100 µg/ml and kills through membrane permeabilization.[27] However, native crotamine was reduced by dithiothreitol and showed enhanced antibacterial activity against three strains of E. coli. Additionally, a time-consuming study found that 25 mcg/mL of reduced crotamine caused a 1 log reduction in E. coli counts.On the contrary, there is evidence that intramolecular disulfide bonds are dispensable for the antibacterial activity of crotamine and have a negative impact on membrane disruption.[27] The acidic PLA2 from the venom of Porthidium nasutum shows a dose-dependent bactericidal effect against S. aureus with a minimum Bactericidal concentration (MBC) of 32 µg/ml.[28] In addition, a basic svPLA2 from the venom of A. halys also shows a significant inhibitory effect against S. aureus and P. vulgaris with an MIC of 11–21 µM each (Fig.4A-B, C-D). On the other hand, mice infected with S. aureus and treated with African Gaboon viper (Bitis gabonica) svPLA2 (3 mg/kg) were cleared of bacteria after 2 weeks and wound closure was accelerated; However, treatment with svPLA2 can cause severe myonecrosis of the skin (Fig. 5A–D, unpublished data). Taken together, the antibacterial property of snake venom is mainly due to PLA2. Furthermore, PLA2 plays a key role in various biological processes, including cell membrane homeostasis, lipid digestion, host defense, signal transduction, and the production of lipid mediators such as eicosanoids and lysophospholipid derivatives, which exhibit diverse and potent biological effects.[29] Furthermore, the antibacterial effectiveness of snake, scorpion and bee venoms was compared with purified svPLA2 enzymes.[21] On the other hand, it has been increasingly shown that sPLA2 have antibacterial activity and are important host defense molecules.[30,31] Elevated levels of group IIA sPLA2 in mammals have been found in inflammatory diseases and are present at levels sufficient to kill gram-positive bacteria.[32,33] High concentrations of these enzymes also have some bactericidal activity against gram-negative bacteria. Similarly, together with other antibacterial proteins, they act as acute phase proteins.[33] The form of tear fluid belonging to group IIA sPLA2 was identified as the main mediator of anti staphylo-coccal activity.[34] Similar to group II sPLA2, group V sPLA2 are bactericidal against Gram-positive bacteria in mammals, suggesting their potential as therapeutic agents against bacterial infections. The mechanisms regulating PLA2 activity are the subject of intensive research, and the control of phospholipid products has long been considered in the treatment of various diseases.[34,35] The discovery of specific inhibitory peptides provides insights into the regulation of intracellular PLA2 activity.[36]

FUTURE IMPACT OF SNAKE VENOM PLA_2S

Snake venom PLA2 are small molecular weight enzymes that affect the physiological and biochemical functions of snakebite victims. They induce a variety of pharmacological events such as cardiotoxicity, neurotoxicity, myotoxicity, hemolysis, hemorrhage and edema. The identification and characterization of target proteins and their detailed mechanisms of pharmacological effects at various cellular and molecular levels have been extensively studied.[37]

Furthermore, the emergence of multidrug resistance represents a serious global threat to public health. Beta-lactams remain the most commonly used antibiotics to treat bacterial infections and their mechanisms of action are also discussed in this review. Bacterial cell walls consist of a complex layer of cross-linked peptidoglycans. In addition, this cell wall includes a basic repeating polysaccharide unit with alternating amino acids, N-acetylglucosamine and N-acetylmuramic acid. Their cross-linking is catalyzed outside the

cytoplasmic membrane by a group of membrane-anchored bacterial enzymes called cell wall transpeptidases.[38] Transpeptidase enzymes utilize a serine in the active site and participate in then catalytic cycle via the anylation/deacylation pathway.

Beta-lactam antibiotics potently inhibit the bacterial transpeptidase enzyme known as penicillin-binding proteins (PBPs). PBPs catalyze the transpeptidase reaction that removes the terminal alanine to form a cross-link with the peptide, giving the cell wall its structural analogues of the natural substrate D-Alan-D-Ala. Transpeptidase forms a lethal covalent penicilloyl enzyme complex that serves to block the normal transpeptidation reaction in the presence of antibiotics.

As a result, the cell wall is weakly cross-linked, which makes growing bacteria very susceptible to cell lysis and cell death.[39] Therefore, new strategies are now required to combat these pathogenic microbial infections. Snake venoms are potent antimicrobial agents that may provide new treatment strategies for immunocompromised populations, including young children and elderly patients. Our previous studies have demonstrated that newly purified proteins/peptides exert broad-spectrum antimicrobial activity with novel mechanisms of action against drug-resistant organisms.[39] The snake protein (y) identified in Naja nigricollis shows a lethal effect on the growth of S. aureus (gram-positive bacteria) and E. coli (gram-negative bacteria). The antibacterial effect of this protein positively increases membrane permeability and alters the integrity of bacterial cells. In addition, (γ) protein plays a key role in binding with lipopolysaccharide (LPS) and lipoteichoic acid (LTA), destabilizing the LPS layer and inhibiting LTA biosynthesis to enhance the bactericidal effect of the cell wall.[40] Cationic peptide (pEM-2) – derived from C-terminus B. asper myotoxin II indicates that electrostatic interactions play an important role in the initial recognition and binding of the peptide to the cell membrane. However, the membrane-damaging effects of the peptide depend on ionic attractive interactions.[41] In contrast, several antibacterial peptides are stand to act by destroying the lipid plasma membrane. They act preferentially on bacteria and are able to interact with and efficiently penetrate anionic phospholipids, while peptides that lyse mammalian cells bind and efficiently penetrate both acidic and zwitterionic phospholipid membranes, mimicking the plasma membranes of these cells. The target membrane varies in structure, length and complexity of the hydrophilic polysaccharide in its outer layer. These parameters influence the ability of peptides to diffuse through the external barrier of cells to reach their cytoplasmic plasma membrane.[42] Recently, model phospholipid membranes were used to study the membrane damage potential of the Taiwan cobra antimicrobial protein CTX3 (Naja naja atra) in egg yolk sphingomyelin vesicles (EYSM). CTX3 guanidination and selective N-terminal alpha-amino acid trinitrophenylation enhanced the membrane damaging effect in EYSM vesicles.[43] However, the positively charged residues of CTX3 play a key role in damaging anionic and zwitterionic phospholipid vesicles. The membrane damage potential of CTX3 was influenced by phospholipid compositions. Therefore, the phospholipid binding capacity and oligomeric structure when binding with lipid vesicles did not closely correlate with the membrane damage effect of native and modified proteins. In addition, it would also be interesting to investigate the mechanisms underlying the wound healing process triggered by venom proteins. Studies to search for new natural antimicrobial agents certainly represent an important part of future drug discovery platforms.

CONCLUSION

For several decades, venom researchers have been working to understand the pharmacology of venom and its use in antidotes. In particular, a report on svPLA2, one of the most important and ubiquitous components of several venoms, has convincingly described these proteins as specific toxins that profoundly affect the hemostasis and homeostasis of vital organs. Interestingly, the antimicrobial nature of svPLA2 and its derived peptides has recently been demonstrated in relation to several bacterial species. Both svPLA2 and sPLA2 (structurally and functionally homologous) were confirmed to have a direct permeabilizing effect on bacterial membranes and have the ability to interact with LPS and components of lipid A. In addition, several short peptides with better antibacterial activity were derived from the C-terminal region of B. Asper myotoxin II, a Lys49 PLA2. Various short peptides (5-13-mers) derived from svPLA2 and sPLA2 inhibitors with modified sequences (by introducing a triple Tyr→Trp substitution) showed significantly increased bactericidal and cytolytic potency and at the same time reduced toxicity to cells. Eukaryotes. in vitro. The bactericidal and toxin-neutralizing properties of short peptides with relatively low toxicity to eukaryotic cells make them promising antimicrobial candidates and warrant further evaluation of their potential in in vivo models. Furthermore, svPLA2 are multifunctional proteins with promising biotechnological applications. The isolation and functional characterization of these enzymes will provide better insights into their mechanisms of action and therapeutic potential, and will also generate new molecular models for future medicine.

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