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Commentary

A brief update on potential molecular mechanisms underlying antimicrobial and wound-healing potency of snake venom molecules

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ABSTRACT

Infectious diseases remain a significant cause of morbidity and mortality worldwide. A wide range of diverse, novel classes of natural antibiotics have been isolated from different snake species in the recent past. Snake venoms contain diverse groups of proteins with potent antibacterial activity against a wide range of human pathogens. Some snake venom molecules are pharmacologically attractive, as they possess promising broad-spectrum antibacterial activities. Furthermore, snake venom proteins (SVPs)/peptides also bind to integrins with high affinity, thereby inhibiting cell adhesion and accelerating wound healing in animal models. Thus, SVPs are a potential alternative to chemical antibiotics. The mode of action for many antibacterial peptides involves pore formation and disruption of the plasma membrane. This activity often includes modulation of nuclear factor kappa B (NF-κB) activation during skin wound healing. The NF-κB pathway negatively regulates the transforming growth factor (TGF)-β1/Smad pathway by inducing the expression of Smad7 and eventually reducing in vivo collagen production at the wound sites. In this context, SVPs that regulate the NF-κB signaling pathway may serve as potential targets for drug development.

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1. Introduction

Hospitals represent a rich environment for several life-threatening bacteria worldwide. For instance, Gram-negative bacterial infection (particularly involving *Pseudomonas* species) is a serious problem in patients hospitalized with cystic fibrosis and burns [1]. The fatality rate is almost 50%, and such infections are difficult to treat with the existing antibiotics [2]. Moreover, sepsis and septic shock are host-derived inflammatory conditions resulting from a systemic response to the bacterial infections [3]. Indeed, sepsis, pneumonia, and other conditions caused by hospital-acquired infections are responsible for at least 48,000 deaths worldwide and cost $8.1 billion to treat infected individuals every year [4].

A number of infections acquired in hospitals are caused by potentially fatal bacteria such as *Staphylococcus aureus*, which can survive for extended periods on medical devices such as intravenous lines and catheter tubes. Nearly 40% of all nosocomial infections involve the urinary tract and use of catheters [5]. Conversely, >70% of nursing home residents are hospitalized every year and exposed to methicillin-resistant *S. aureus* (MRSA) [6]. A previous study reports that 4.4% of all *S. aureus* strains are MRSA among children treated with flucloxacillin for noninfected atopic

Abbreviations: SVPs, snake venom proteins; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci; CT, ultrasound and computed tomography; MRI, magnetic resonance imaging; MDR, multidrug resistant; PLA_2s, phospholipase A_2s; LAAO, L-amino acid oxidases; SVMP, snake venom metalloproteinases; ALT-C, alternagin-C; HUVEC, human vein endothelial cell; VEGF, vascular endothelial growth factor; MMPs, matrix metalloproteinases; TNF, tumor necrosis factor; IFN-γ, interferon-γ; TGF-β, transforming growth factor-β; TLRs, Toll-like receptors; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein 1; MIP-1α, macrophage inflammatory protein 1α; CTGF, connective tissue growth factor; NF-κB, nuclear transcription factor-kappa B; CDK, cyclin-dependent kinase; GM-CSF, granulocyte–macrophage colony-stimulating factor; CaTx-II, *Crotalus adamanteus* toxin-II.

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eczema. A 15–20% prevalence of fusidic acid resistance and MRSA in children (infancy to school age) has been reported for infected atopic eczema [7]. Previous reports related to decubital ulcers and inflammatory skin diseases with erosive lesions and venous leg ulcers mention the key role of MRSA [8]. In addition, severe infections may also complicate 0.25–4% of major cardiac surgical procedures, causing death and morbidity, leading to higher costs [9,10]. Generally, in diabetes mellitus and AIDS, a normally mild infection can rapidly become life-threatening [11]. In the case of diabetic foot ulcers caused by *S. aureus*, which produces the Panton–Valentine leukocidin and toxic shock syndrome toxin 1, persistent tissue damage and inflammatory response are observed. As a result, the inflammation may be the source of ongoing tissue damage [11,12]. Exotoxins are actively secreted proteins produced by virulent strains of *S. aureus* and *Streptococcus pyogenes* [13], which cause tissue damage or dysfunction via diverse mechanisms. It is estimated that half of all *S. aureus* strains develop resistance to antibiotics such as methicillin. The emergence of vancomycin-resistant *Enterococcus* species may accelerate the spread of vancomycin resistance genes through plasmids to other species. The diagnosis and treatment perspectives include technological advances for diagnosis by blood cultures, tissue swabs with culture, needle aspiration, X-ray, ultrasound, and computed tomography (CT) scan or magnetic resonance imaging (MRI) screening, depending on the clinical manifestations. However, these techniques fail to detect microorganisms sometimes [10] and subsequently delay proper treatment.

Currently, there is an urgent need for an effective treatment regimen to prevent further emergence of multidrug-resistant (MDR) microorganisms. Furthermore, MDR significantly increase the risk of these infections; new antibacterial sources and agents are needed. Moreover, antimicrobial resistance is recognized as one of the greatest threats to human health worldwide [14]. Novel, promising sources of antimicrobials include antimicrobial peptides derived from various biological sources. For the past 20 years, several antimicrobial peptides have been isolated from plants, insects, and animals [15,16] as defense molecules, playing a vital role in innate immunity [17]. Animals are significant producers of antimicrobial peptides, most of which have been identified from invertebrate and vertebrate species [18].

This review focuses on a wide variety of snake venom proteins (SVPs) and peptides with antimicrobial effects and wound-healing accelerating properties. The most interesting biological and pharmacological properties are linked to a variety of enzymes such as phospholipase A_2 (PLA_2), metalloproteases, serine proteases, L-amino acid oxidases (LAAO), esterases, and other proteins such as disintegrins [19]. Recently, a snake (*Bothrops alternatus*) metalloproteinase called alternagin-C (ALT-C) has received attention. It is a disintegrin-like, cysteine-rich protein that promotes wound healing, induces increased type I collagen deposition and fibroblast density, and reduces inflammation in rats within 7 days [20]. Snake neurotoxins, myotoxins, cardiotoxins, and cytotoxins induce different toxic effects [21]. Some of these SVPs are not only toxic but also excellent antimicrobial agents [22].

2. Antimicrobial properties of SVPs

Thirty different snake venoms were assayed against Gram-negative and Gram-positive bacteria by a disk-diffusion method [23]. Purified LAAO (LAO1 and LAO2) from *Pseudechis australis* venom shows promising antibacterial activity that is, respectively, 70 and 17.5 times more effective on a molar basis than tetracycline is against *Aeromonas* [23]. A more recent study reports that among 14 Elapidae and eight Viperidae venoms tested for antibacterial effects, *Bothrops moojeni* and *Bothrops jararacussu* venoms inhibit the growth of *Streptococcus mutans*, a principal agent involved in dental caries [24]. Furthermore, *Naja naja* venom, as well as purified peptides, exhibits potent antibacterial activity against both Gram-positive and Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *S. aureus*, and *Bacillus subtilis*. The most potent peptides target Gram-negative bacteria at concentrations of 100 μg/ml [25]. In addition, crude venoms from Viperidae species demonstrate significant inhibitory effect; interestingly, *Calloselasma rhodostoma* venom generates the largest inhibition zones against *S. aureus* (minimum inhibitory concentration (MIC) of 125 μg/ml) [26]. The antimicrobial profile of four different Viperidae venoms (*Agkistrodon rhodostoma*, *Bothrops atrox*, *Bothrops jararaca*, and *Lachesis muta*) against 10 drug-resistant, Gram-positive and Gram-negative bacteria has also been reported [27]. To date, Viperidae venoms represent a rich source of proteins and peptides that are yet to be studied. With these and other studies, snake venoms may represent valuable resources for the development of novel human therapeutics in future [28].

Snake venoms and, in particular, their PLA_2s exhibit stronger antibacterial activity even at low concentrations [29,30]. Type IIA secretory PLA_2s are endogenous antibiotic-like proteins which exert antibacterial activity [31–33]. One specific example includes crotamine from *Crotalus durissus terrificus*, which inhibits several strains of *E. coli* (MIC ranges of 25–100 μg/ml) via membrane permeabilization [34]. Importantly, crotacetin is a novel snake venom C-type lectin isolated from *C. durissus terrificus*, sharing homology with convulxin, which also exhibits antimicrobial activity against both Gram-positive and Gram-negative bacteria [35]. A PLA_2 myotoxin from the venom of this South American rattlesnake is structurally close to beta-defensin antimicrobial peptides found in other vertebrates [34]. Myotoxin has been found to exhibit a significant antibacterial effect independent of PLA_2 enzymatic activity [36]. More interestingly, a PLA_2 derived peptide exhibits fungicidal activity against *Candida albicans* [37]. Cationic peptides designed from amino acids (KKWRWWLKALAKK) 115–129 of a Lys49 PLA_2 in *Bothrops asper* venom exhibit both bactericidal and anti-endotoxic properties [29]. Several synthetic peptides can be derived from parent SVPs that are also PLA_2s. These small novel molecules not only display broad-spectrum activities but are also nontoxic and yet increase the bactericidal potency compared with the parent molecules.

The peptide family termed "cathelicidins" with a common proregion (cathelin domain) was first identified in bone marrow myeloid cells of mammals [38]. Therefore, they are also known as "myeloid antimicrobial peptides" or MAPs. Cathelicidins are a group of antimicrobial peptides, varying in amino acid sequence, structure, and size [39]. Cathelicidins have been found in humans and other species including cattle, horses, pigs, sheep, goats, chickens, rabbits, birds, and some fish [40]. Recently, cathelicidin-based antimicrobial peptides were identified from *Bungarus fasciatus* venom [39,41,42] and are the first to be reported in reptiles. The mode of action of many antibacterial peptides is disruption of the plasma membrane. These studies have demonstrated that these peptides act preferentially on bacteria by efficiently permeating anionic phospholipid bilayers, whereas peptides that lyse mammalian cells efficiently bind and permeate both acidic and zwitterionic phospholipid membranes, mimicking the plasma membranes of these cells. With respect to the target membrane, antimicrobial peptide activities primarily depend on the structure, length, and complexity of hydrophilic polysaccharides found in the outer layer of the membrane. These parameters affect a peptide's ability to diffuse through the cell's outer barrier and reach its cytoplasmic membrane [43]. However, these peptides may have a direct effect on microorganisms by damaging or destabilizing the bacterial, viral, and fungal membrane or by acting on unknown targets. Interestingly, antimicrobial peptides also play a key role in

innate immunity and the inflammatory response [44]. However, these snake-derived cathelicidin peptides may be used as models for developing novel therapeutic drugs with antimicrobial properties.

3. Structure and activity relationship of SVPs

Snake venom PLA_2s are an interesting protein superfamily that catalyze the hydrolysis of the 2-acyl bond of cell-membrane phospholipids, releasing arachidonic acid and lysophospholipids. These proteins are found abundantly in nature. Snake venom PLA_2s have a very high content of disulfide bonds, relatively low molecular mass (10–20 kDa) and similar three-dimensional structures (Fig. 1A and B). The PLA_2s from snake venoms belong to either group I or II [45–47]. Group I consists of PLA_2s from Elapidae and Hydrophidae venoms that contain 120 amino acids with seven disulfide bonds. These enzymes may contain a surface cysteine loop (63–37) called the pancreating or elapidic loop [47]. They are calcium dependent and contain an aspartic acid at position 49 (Asp49-PLA_2 or D49-PLA_2) [48–50]. Group II PLA_2s are derived from Crotalidae and Viperidae venoms, containing 125 amino acids and seven disulfide bonds with no elapidic loop. These molecules possess an additional tail on the carboxy-terminal region and an extra disulfide bond close to His48 (Cys50–Cys138) [46]. His48 has an important catalytic role in the activity of PLA_2, together with Tyr52 and Asp99. Alkylation of His48 with p-bromophenacyl bromide (p-BPB) completely or partially abolishes catalytic and other pharmacological activities [48,50]. Thus, His-modified enzymes are suitable for investigating the effect of their enzymatic activity on the PLA_2 pharmacological profiles [48]. Some snake venom PLA_2s (group IIA), such as vipoxin from *Vipera ammodytes meridionalis*, possess an Asp49 PLA_2 that interacts with Ca^{2+}. Interestingly, the canonical Ca^{2+}-binding loop formed by Trp28, Gly30, Gly32, and Asp49 is not disturbed by alkylating His48, and essentially Ca^{2+} protects the enzyme from inactivation. Alternatively, this enzyme contains a hydrophobic channel composed of Gly30, His48, Tyr52, Trp73, and Asp99 [51,52]. Furthermore, the structural determinants of this toxic effect are experimentally mapped to the C-terminus (residues 115–129), which is antibacterial and combines both cationic and hydrophobic aromatic amino acid residues from Lys49 and Asp49 myotoxic PLA_2s of *B. asper*. However, most cationic and hydrophobic groups of snake proteins and peptides exhibit antibacterial action against a wide range of human pathogens (see Table 1).

4. SV proteins/peptides as novel candidates for wound healing

Antibiotic resistance has become increasingly prevalent within dermatology, specifically for wound healing [53]. Antimicrobial peptides are considered new antimicrobial agents with low resistance development reported against them. One of our previous studies evaluated the anti-staphylococcal activity of SVPs and peptides in vitro and in vivo, some of which are promising candidates. Currently, the antibacterial properties of different types of SVPs such as PLA_2s, metalloproteinases, and peptides have emerged from several reports in recent years [54]. Local application of SVPs completely clears *S. aureus* infection with a 14-day treatment regimen, which is a better outcome than with commercial antibiotics [55]. Another SVP isolated from Eastern diamondback (*Crotalus adamanteus*) venom exhibited not only antimicrobial but also wound-healing properties. This protein, *C. adamanteus* toxin-II (CaTx-II), not only exerts potent bactericidal effects on *S. aureus* at a dose of 7.8 μg/ml but also modulates the activation of nuclear factor kappa B (NF-κB) in skin wound repair in an established mouse model [56]. Therefore, SVPs and peptides will be useful in developing antimicrobial and wound-healing agents in future [54]. Oxynor is a synthetic therapeutic agent (β-taipoxin) discovered from the venom of the Australian taipan snake (*Oxyuranus s. scutellatus*) that promotes wound healing. Oxynor (100 μg/ml) showed no toxic effect on ischemic skin wounds in rats and is under clinical development for wound healing [57].

Several snake venom compounds are effective against bacterial infections and promote wound healing. Fibrin glue obtained from snake venom is used as an alternative suturing agent that reduces

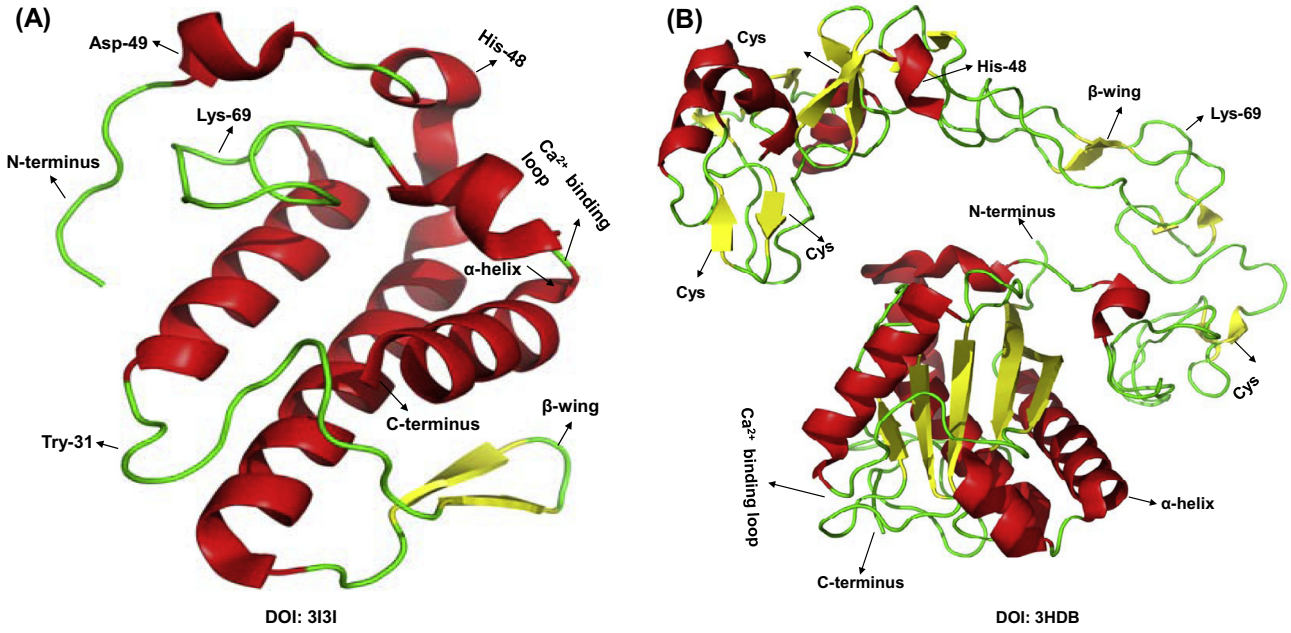

Fig. 1. (A) A comparison between apo "structure of the enzyme with no ligand" and complex structures of bothropstoxin-I reveals the role of Lys122 and the Ca^{2+}-binding loop region for the catalytically inactive Lys49-PLA_2s. Antibacterial effects based on the catalytic and hydrophobic nature of snake proteins (PDB, DOI: 3I3I). (B) *Agkistrodon acutus* (AaHIV), a P-III-type snake venom metalloproteinase (SVMP), consists of metalloproteinase/disintegrin/cysteine-rich (MDC) domains homologous to disintegrin and metalloproteinase (ADAM) family proteins (PDB, DOI: 3HDB).

Table 1
Summary of the snake venom-derived peptides/candidate molecules under preclinical/clinical development.

Proteins/peptides	Snake species	Properties	Mechanism(s) of action	References
Viperatoxin (VipTx-II)	*Daboia russelli russelli* (Indian Russell's viper)	MICs 6.25 μg/ml on *Burkholderia pseudomallei/S. aureus*, MICs 12.25 μg/ml on *Proteus vulgaris*	Pore formation and causes cell wall membrane damage on bacteria	[98]
Peptide	*Naja naja* (Indian cobra)	Antimicrobial (Gram-negative bacteria at 100 μg/ml)	–	[25]
Cathelicidin (Hc-CATH)	*Hydrophis cyanocinctus* (Sea snakes)	MICs 0.16 μM (*Shigella dysenteriae*), *Klebsiella pneumoniae* (MICs 8 μM)	Membrane damage or cellular inclusion efflux induced by Hc-CATH	[99]
Omwarpin	*Oxyuranus microlepidotus* (Inland taipan) (25 kDa)	MICs 2–10 μg (Gram-positive bacteria)	–	[100]
Cationic peptides (Lys49-PLA_2)-myotoxin II KKWRWWLKAL AKK (115–129 AA)	*Bothrops asper* (Pit viper)	MMCs 1 μg/ml (*K. pneumoniae*), 100 μg of pEM-2 peptide-treated mice were cleared of peritonitis induced by *Salmonella enterica*	Membrane-permeabilizing action of *S. aureus* bacteria	[29]
Cationic peptides (Lys49-PLA_2)-myotoxin II	*Bothrops asper* (Pit viper)	High microbicidal potency against *S. aureus/Salmonella typhimurium*	Functionally interacts with lipopolysaccharide (LPS) in a chimeric bacteria model	[101,102]
Cathelicidin (BF)	*Bungarus fasciatus* (Banded krait)	MICs 0.6 μM (*Escherichia coli*)	Displays potent, broad-spectrum, salt-independent antimicrobial activities	[42]
CaTx-II (protein)	*Crotalus adamanteus* (Eastern Diamondback Rattlesnake)	MICs 7.8 μg/ml (*S. aureus*), *B. pseudomallei*/*Enterobacter aerogenes* (MICs 15.6 μg/ml)	Membrane-damaging effects/pore formation	[56]
Phospholipase A1 inhibitor	*Python reticulates* (Python serum)	MICs 3.125 μg/ml (*S. aureus*/*B. pseudomallei*) peptide PIP-18 [59–76] showed wound healing (50 mg/kg body weight) in mice model of *S. aureus* infection	Membrane disintegration/wound repair	[103]
Alternagin C (ALT-C PEP)	*Bothrops alternatus* (Crossed pit viper)	10, 50, 100 ng of ALT-C rat increased fibroblast density/collagen deposition at day 7 in rats	Angiogenesis and growth modulation induced by snake venom disintegrin-like, cysteine-rich protein	[20]
Cathelicidin-BF30	*Bungarus fasciatus* (Banded krait)	Killing effects on *P. aeruginosa/S. aureus* (2 logs within 6 min); BF-30 treated burn/acute infection rat model showed potent reduction of bacteria/healing at 0.75, 3, 12 mg/kg/day	Cell debris, cytoplasmic leakage	[104]
Crotamine (toxin)	*Crotalus durissus terrificus* (South American rattlesnake venom)	Peptide (10 μg/well) caused marked permeabilization of *S. aureus*	Peptide that selectively targets microbial or abnormal host cells	[105]
Crotamine (basic polypeptide-myotoxin)	*C. durissus terrificus*	Antifungal activity on *Candida* spp., *Trichosporon* spp., *Cryptococcus neoformans* (12.5–50 μg/ml)	Ultrastructural alteration of *Candida albicans*, low toxicity/no	[106]
Crotamine (basic myotoxin)	*C. durissus* (South American rattlesnake)	Strong antibacterial effect against several strains of *Escherichia coli* (MICs 25–100 μg/ml)	Killing of bacteria by membrane permeabilization	[34]
Cathelicidin (NA-CATH)	*Naja naja* (Chinese cobra)	Potent antimicrobial action on *Burkholderia thailandensis* at 3.6 μg/ml)	Evades destruction of Gram-negative bacteria by NA-CATH	[107]
Synthetic peptides (ATRA-1,-2, 1A); NA-CATH–ATRA1-ATRA)	*Naja naja* (Chinese cobra)	NA-CATH–ATRA1-ATRA inhibited biofilm production by *S. aureus* (0.51 μg/ml)	Anti-biofilm tools may be a useful template for the treatment of chronic wound infections	[108]
Lectin (BIL)	*Bothrops leucurus* (Whitetail lancehead)	Effective antibacterial activity on *S. aureus*, *E. faecalis*, *Bacillus subtilis* (31.25, 62.25, 125 μg/ml)	Secondary structure possess α-helix and β-sheet responsible for antibacterial effects	[109]
Oxynor (synthetic peptide)	*Oxyuranus scutellatus scutellatus* (Australian taipan)	Mice wound (4 mm) treated with active domain of Oxynor (500 μg/mouse)/β taipoxin (100 μg/ml for PC12 cells) for 7 days	Nontoxic β-taipoxin showed mitogenic action on cells/wound healing in mice FDA approved for human non-healing wounds	[57]
Captropirls (Bj-BPP-10c)	*Bothrops jararaca* (South American snake)	Bradykinin-potentiating peptides	Inhibitor of angiotensin-converting enzyme (ACE)	[110]
Echistatin	*Echis carinatus* (Saw-scaled viper)	Antagonists	Motif binds to glycoprotein IIb/IIIa integrin receptors	[111]
Mambalgins (Peptide)	*Dendroaspis polylepis polylepis* (Black mamba)	Peptide showed potent analgesic effects as powerful as morphine	Venom peptides target acid-sensing ion channels to alleviate pain	[85]
α-Neurotoxin	*Ophiophagus Hannah* (King cobra)	Hannalgesin (painkiller)	Neurotoxin produces analgesia (16–32 ng/g, i.p) without any neurological deficits	[112]
Dimeric complex (MiTx)	*Micrurus tener tener* (Texas coral snake)	Analgesic (painkiller)	Induces pain-like behavior in mice	[113]
Drug (CB24)	*C. adamanteus* (American diamondback rattlesnakes)	Anticancer effects	Targets and kills cancer cells	[114]
Protein ACTX-6/ACTX-8	*Agkistrodon acutus* (Sharp-nosed viper)	Anticancer potency	Induces apoptosis in cervical cancer cells	[115]

adherence, strengthens the wound site [58], has an adhesive effect on organ elasticity, stimulates the formation of more granulating tissue in uterine scars [59], and accelerates healing. In addition, ALT-C, a disintegrin-like cysteine-rich protein isolated from *B. alternatus* venom, induces angiogenesis. Wounded rats treated with Natrozol gel alone showed accelerated wound healing with 10, 60, and 100 ng of ALT-C for 1, 3, 5, and 7 days [20]. Thus, this scientific study provides sufficient evidence for the potential of SVPs in the development of novel therapeutics for wound repair.

5. Cysteine-rich SVPs induce wound healing

Integrins form a family of cell-surface adhesion receptors that can mediate both cell to cell and cell to extracellular matrix (ECM) interactions. The α2β1 integrin is a chief collagen receptor that plays a key role in the adhesion of normal and cancerous cells to the ECM [60]. Families of small integrin-binding proteins called disintegrins are found in snake venoms, which strongly inhibit integrin-mediated cell adhesion and migration [61]. For instance, alternagin is a protein isolated from *B. alternatus* venom. It is synthesized as a precursor belonging to the PIII class of snake venom metalloproteinases (SVMPs). ALT-C is a disintegrin-like cysteine-rich domain released from alternagin and is a promising collagen inhibitor that blocks α2β1 integrin [61,62]. An earlier study showed that ALT-C strongly stimulates the proliferation of human vein endothelial cell (HUVEC) both in vitro and in vivo by upregulating the expression of growth factors and their receptors, including vascular endothelial growth factor (VEGF) and VEGF receptor 2 (VEGFR-2) [63,64]. In addition, ALT-C increases myoblast proliferation by modifying gene expression that includes the *MHC* gene [65,66]. ALT-C also modulates matrix metalloprotease (MMP) activity and expression in an in vivo skeletal muscle regeneration model [66]. A later study revealed that ALT-C and ALT-C PEP (amino acids 1–196) can also induce angiogenesis in rat skin with wounds [67]. However, these results confirm the role of α2β1 integrin in the healing process, which may also be useful in further developing therapeutic strategies for wounded skin repair and regeneration [20]. Indeed, subsequent studies prove that ALT-C significantly enhances the expression of type I/type III collagen

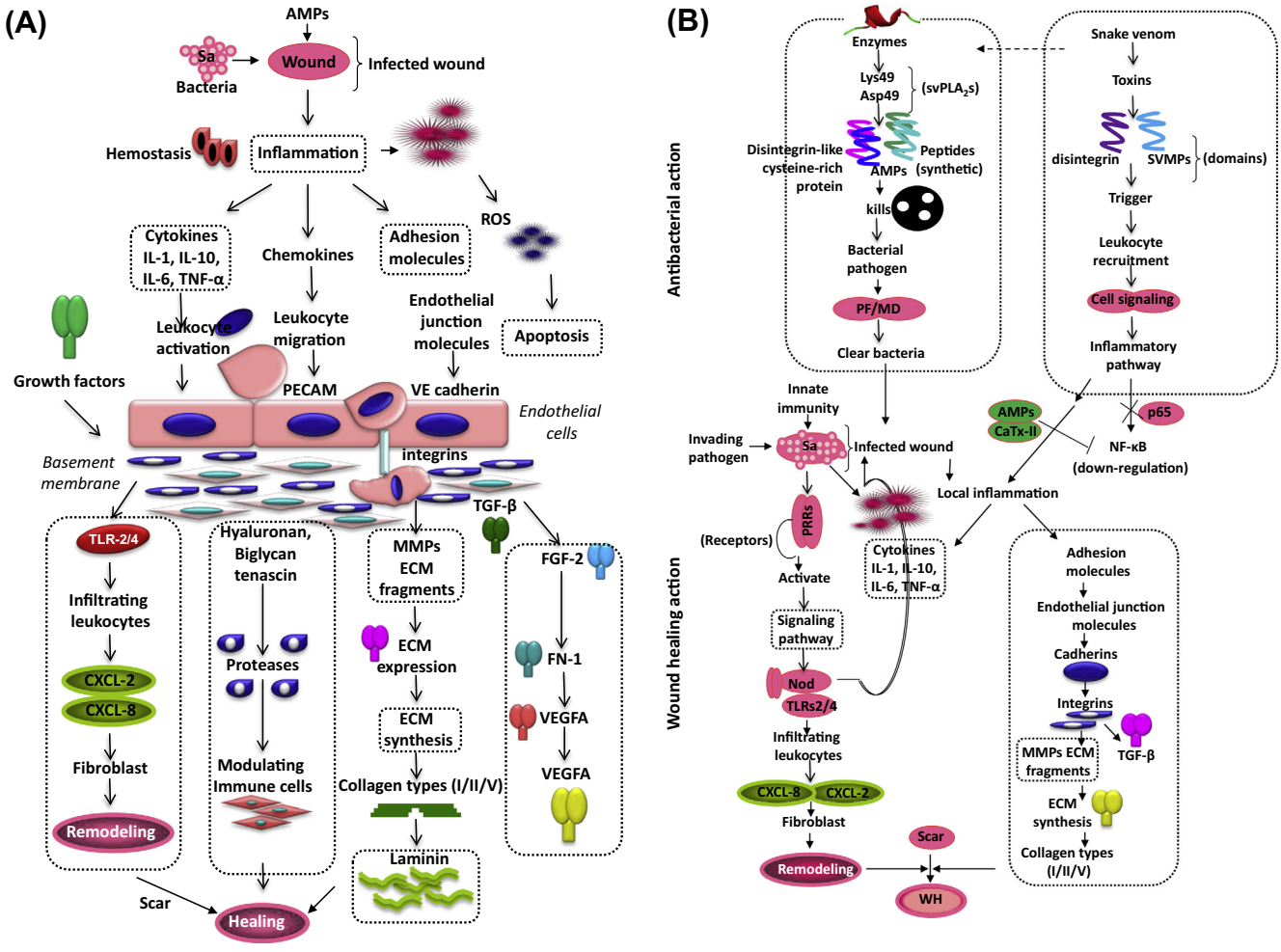

Fig. 2. (A) A flowchart representing the diverse mechanism(s) of action for extracellular matrix (ECM)-mediated activation of immune cells. (B) Snake venom molecules protect against invading pathogens and accelerate wound repair. Snake venom metalloproteinase (SVMP) triggers inflammatory conditions by stimulating edema, leukocyte recruitment to tissues, and release of inflammatory mediators that cause a number of systemic/local inflammation links to cell signaling inflammatory pathways. Snake venom molecules such as toxic proteins/phospholipase A_2 enzymes (Lys49/Asp49), including disintegrin-like cysteine-rich proteins, and short synthetic peptides derived from svPLA$_2$s show promising antibacterial action against *S. aureus* and accelerate potent wound healing in rodent models. The immune system recognizes pathogens through pattern recognition receptors (PRRs), which activate unique signaling pathways of TLRs4/NOD-like receptors and leads to local inflammation at the infected wound site. Infiltrating leukocytes activate the release of chemokines (CXCL-8/CXCL2), fibroblast/adhesion molecules that help promote collagen production crucial for wound healing. Symbols: Lys49/Asp49-PLA$_2$s, lysine49/aspartate49 phospholipase A_2s enzyme; svPLA$_2$, snake venom phospholipase A_2; PF, pore formation; MD, membrane damage of bacterial cell wall; Sa, *Staphylococcus aureus*; PRRs, pattern recognition receptors; Nod, Nucleotide-binding oligomerization domain receptor; TLR2/4, Toll-like receptors 2/4; WH, wound healing; ROS, reactive oxygen species.

and transforming growth factor (TGF)-α in a rat model for skin wounds [20]. Further sequence modification(s) of ALT-C/ALT-C PEP may not only further enhance the cytotoxic effects of these proteins on bacteria but also significantly decrease their potential toxic effect on eukaryotic cells [68].

6. Activation of NF-κB by SVPs in wound healing

Wound healing is a complex process involving interactions among a variety of different cell types [69,70]. In a severe wound and chronically inflamed tissues, inflammatory cytokines and proteases (particularly MMPs) are released by extravasation, and activated tissue-resident cells that ultimately modify the ECM [71]. Inflammation progresses via the release of diverse pro-inflammatory cytokines, including interleukin (IL)-1, TNF-α, interferon-gamma (IFN-γ), IL-12, IL-18, and the granulocyte–macrophage colony-stimulating factor (GM-CSF). The inflammatory process is resolved by anti-inflammatory cytokines such as IL-4, IL-10, IL-13, IFN-α, and TGF-β, and other molecules such as annexin-A1 (Fig. 2A). The signal transduction pathways of these cytokines have been studied extensively, ultimately activating transcription factors such as NF-κB, Smad, and STATs [72]. The activation of the pro-inflammatory NF-κB protein leads to cell damage and death in skin wound tissues [72–74]. In corroboration with earlier studies, we also noted activation of NF-κB in skin wounds [56]. We found enhanced nuclear localization of p65 in wound control mice, indicating its involvement in this process. By contrast, treatment with *C. adamanteus* toxin-II (CaTx-II) causes downregulation of NF-κB and more rapid wound repair (Fig. 2B). We hypothesize that NF-κB plays a negative role in response to CaTx-II treatment of wound healing [56]. NF-κB is an important transcription factor with a vital role in several cellular activities such as proliferation, activation of immune cells, and development of T/B lymphocytes. Interesting cross talk may occur between NF-κB and TGF-β1/Smad signaling cascades during skin wound healing [75–79]. The TGF-β isoforms (TGF-β1, TGF-β2, and TGF-β3) are synthesized as latent precursors, which are regularly secreted as a complex with the latent TGF-β-binding protein that is removed by extracellular proteolytic cleavage (Fig. 3). Active TGF-βs then exert their biological function by binding to a heteromeric receptor complex, which consists of type I and type II receptors that are also serine–threonine kinases. TGF-βs bind with high affinity to a non-signaling type III receptor that mainly presents TGF-β to the type II receptor [80]. Thus, TGF-βs act in a similar manner to wound cytokines and are thus among the most studied molecules in the wound-healing process.

7. Venom-based drugs and therapeutics potential

Snake venom is not only toxic but also used to treat various human ailments, especially their secreted peptides/enzymes, as evidenced in current medical practice [81]. The peptide-based antihypertensive life-saving drug captopril was discovered in 1970 from the venom of a Brazilian viper, *B. jararaca* [82]. This viper venom drug is an angiotensin-converting enzyme (ACE) inhibitor used to treat hypertension [83]. Currently, several venom-based drugs are being developed; for example, a pain-relieving agent called hannalgesin is 20–200 times more effective than morphine [84] and is also used as an anti-inflammatory agent. Hannalgesin is a toxin-derived drug obtained from king cobra (*Ophiophagus hannah*) venom. Peptides with analgesic effects have also been described from venom of the black mamba (*Dendroaspis polylepis polylepis*) [85]. Australian elapid snake venoms effectively prevent bleeding, for example, textilin-1 (code number Q8008), haempatch™ (Q8009), and CoVase™ (V0801). Crotoxin is a protein derived from the South American rattlesnake [84], and other molecules may be an attractive option for treating cancer [86,87]. The United States Food and Drug Administration (FDA) have approved drugs derived from cone snail venoms (ω-conotoxin CVID) that can be used to treat chronic pain [88]. A recent study urges the use of various venoms as a platform for human drugs, thereby emphasizing the rapid translation of venom toxins into therapeutics [89]. The potential of toxin-derived molecules and in particular some of the disulfide-rich venom peptides are under clinical trials [90].

8. Concluding remarks

Although the bites of certain snakes can be deadly, their venoms contain diverse components of medical, biotechnological, and pharmaceutical importance [91]. Proteins and peptides derived from natural toxins found in animal venoms provide an invaluable template for developing new drugs to treat human disorders. Toxicology and clinical safety are the most common reasons for the failure of these molecules during development and clinical studies [92,93]. The venom-derived drugs currently under development must be passed through preclinical evaluation/clinical trials to examine their therapeutic efficacies before translation [94]. The medical industry is currently focusing on disulfide-rich peptides, as broad-spectrum molecular tools to treat diverse clinical disorders or infections [95,96]. These therapeutic peptides may be useful for oral delivery, as some peptide drugs can breach the

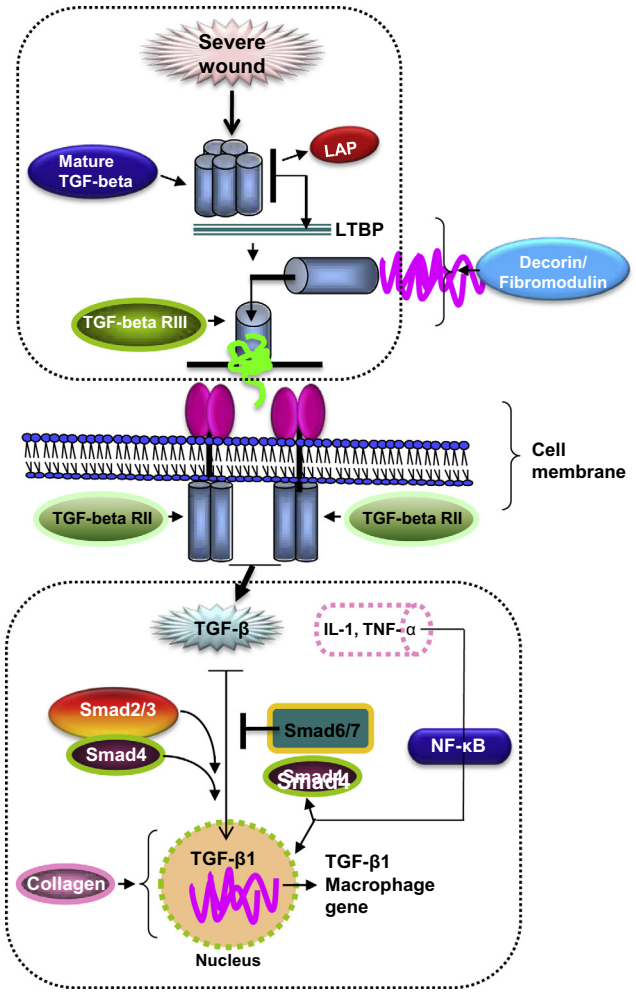

Fig. 3. The process of wound healing involves potential cross talk between TGF-β and NF-κB signaling pathways.

blood–brain barrier and be translocated across cell membranes. Thus, intracellular targets [89] can be considered with high potency and specificity [97]. In addition, these peptide molecules highlight the importance of peptide drugs, which are of great potential despite the several challenges that lie ahead.

SVPs and peptides may be potentially useful, novel antibiotics to combat infections, including those caused by antibiotic-resistant bacteria such as *S. aureus* and *Enterococcus*. For example, the disintegrin-like, cysteine-rich snake protein alternagin is a potent inhibitor of collagen-induced adhesion by blocking $\alpha 2\beta 1$ integrin, cytokines, and TGF-β influences during wound healing. Annexin-A1 (ANXA1) is an important regulator of wound healing and may act in coordination with both NF-κB and TGF-β1/Smad signaling pathways. Molecular cross talk is often seen between the NF-κB and TGF-β1/Smad signaling pathways during skin wound healing. Snake venom contains various groups of proteins and peptides that exhibit antibacterial activity against a wide range of human pathogens. Some of these multifunctional proteins also promote wound healing in well-established animal models by modulating NF-κB activation. Ultimately, small venom-derived candidate molecules such as peptides may serve as useful tools to develop novel anti-inflammatory and wound healing therapeutics.

Conflict of interest

The authors have declared that there is no potential conflict of interest.

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