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Review

Animal venoms as antimicrobial agents

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ABSTRACT

Hospitals are breeding grounds for many life-threatening bacteria worldwide. Clinically associated gram-positive bacteria such as *Staphylococcus aureus*/methicillin-resistant *S. aureus* and many others increase the risk of severe mortality and morbidity. The failure of antibiotics to kill various pathogens due to bacterial resistance highlights the urgent need to develop novel, potent, and less toxic agents from natural sources against various infectious agents. Currently, several promising classes of natural molecules from snake (terrestrial and sea), scorpion, spider, honey bee and wasp venoms hold promise as rich sources of chemotherapeutics against infectious pathogens. Interestingly, snake venom-derived synthetic peptide/snake cathelicidin not only has potent antimicrobial and wound-repair activity but is highly stable and safe. Such molecules are promising candidates for novel venom-based drugs against *S. aureus* infections. The structure of animal venom proteins/peptides (cysteine rich) consists of hydrophobic α-helices or β-sheets that produce lethal pores and membrane-damaging effects on bacteria. All these antimicrobial peptides are under early experimental or pre-clinical stages of development. It is therefore important to employ novel tools for the design and the development of new antibiotics from the untapped animal venoms of snake, scorpion, and spider for treating resistant pathogens. To date, snail venom toxins have shown little antibiotic potency against human pathogens.

Contents

Abbreviations: Bp, Burkholderia pseudomallei; CDC, Centers for Disease Control and Prevention; NNIS, National Nosocomial Infections Surveillance; MRSA, Methicillin-resistant *S. aureus*; VRSA, Vancomycin-resistant *S. aureus*; PBP2a, Penicillin binding protein 2a; IL-2, Interleukin-2; IL-4, Interleukin-4; IL-6, Interleukin-6; IL-12, Interleukin-12; IL-17, Interleukin-17; TNF-α, Tumor necrosis factor-alpha; Th1, Type 1 T helper cell; Th17, Type 17 T helper cell; TLRs, Toll-like receptors TLR2/TLR4; LPS, Lipopolysaccharides; IL-1R, Interleukin-1 receptor; MyD88, Myeloid differentiation primary response 88; NF-kB, Nuclear factor kappa-light chain enhancer of activated B cell; MMPs-2, matrix metalloproteinases 2; MMPs-9, matrix metalloproteinases 9; MMPs-10, matrix metalloproteinases 10; AMPs, antimicrobial peptides; NA-CATH, *Naja naja* cathelicidin; ALT-C PEP, Alternagin C peptide; Lys49-PLA_2, Lysine49 phospholipase A_2; FDA, Food and Drug Administration; PLA_2, Phospholipase A_2; LAAO, L-amino acid oxidase; CAMPs, cationic antimicrobial peptides.

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1. Introduction

Hospitals are breeding grounds for many life-threatening bacteria worldwide. For example, the gram-negative bacterium *Burkholderia pseudomallei* causes melioidosis [1] and is involved in bacteraemia, necrotizing hepatitis, and splenitis. Severe acute diseases have been observed [2–4] in endemic areas of Southeast Asia, Oceania and other parts of tropical/subtropical regions of the world. *B. pseudomallei* is an important septic mediator that also causes severe inflammation [5,6] and often fatal organ failure [7]. Gram-positive *Staphylococcus aureus* is also a major source of mortality in medical facilities, causing a wide variety of infections that include skin infections [8], toxic shock syndrome [9], psoriasis [10], pneumonia [11,12], surgical site infections (~157,500 cases) [13] and general nosocomial infections (~250,000 cases) each year in the United States [14]; moreover, it was the first pathogen to be detected in cystic fibrosis, a genetic suppurative disease. In 2011, there were an estimated 648,000 patients with 721,800 medical device-associated infections in acute care hospitals in the US [15]. More than 70% of nursing home residents are hospitalized every year and exposed to methicillin-resistant *S. aureus* (MRSA) [16,17]. The carriage of clinically associated MRSA increases the risk of severe mortality and morbidity [18]. Several studies have also shown that the overuse of antibiotics, such as vancomycin, can lead to vancomycin-intermediate (VISA)/vancomycin-resistant (VRSA) strains indicative of emerging drug resistance [19,20]. However, the high number of MRSA strains is mainly due to an acquired penicillin-binding protein 2a (PBP2a) encoded by the *mecA* gene [20]. Structural-functional studies have clearly revealed that PBP2a possesses both transglycosylase and transpeptidase activity, thus conferring resistance to all β-lactam antibiotics [21]. More than 90% of *S. aureus* strains are resistant to β-lactam antibiotics [22]. Common problems with *S. aureus* infections include increased prevalence of delayed wound healing due to an ageing population, as currently found in Singapore [23–25], ultimately leading to higher healthcare costs [26]. The estimated cost associated with the management of a chronic ulcer wound is as high as SGD 45,000, in addition to a decreased quality of life, restricted mobility, loss of limb, severe pain, and a hefty socio-economic impact. Non-healing chronic skin wounds are dramatically increasing healthcare costs that are estimated at $25 billion annually in the United States [27].

The failure of antibiotics to kill certain pathogens highlights the urgent need to develop novel and potent, yet less toxic, agents from natural sources. Snake venoms hold promise as a rich source of chemotherapeutically attractive agents against *S. aureus* and other pathogens [28,29]. Currently, several promising classes of natural venoms and synthetic antimicrobial peptides (AMPs) have demonstrated broad spectrum antimicrobial activities against bacteria, viruses, fungi and parasites [30,31].

2. Animal venoms as rich sources of antimicrobials

Antibiotic discovery and their subsequent clinical use peaked during the golden age (1940–1970) and has helped to steadily increase human life expectancies [32]. Currently, the clinical scenario reveals an ever increasing and alarming number of multidrug resistant (MDR) pathogenic bacteria. Clearly, such resistance limits the efficacy of many existing antibiotics. Recently, a large number of drugs have been introduced into the clinical pipeline and are at various phases of clinical trials or remain at an experimental stage. It is obvious that the discovery of novel antimicrobials is essentially required to combat pathogens now, and well into the future.

Snakes are the most venomous reptilian group known. Venomous snakes reside throughout several regions, especially in the rural tropics. The incidence of snakebite-caused morbidity/mortality (1300–50,000) is a leading threat to public health [33,34]. Roughly 3000 species of snakes are found worldwide, of which 600 species are venomous and 200 of these are considered medically important [35,36].

These venomous snakes belong to the families *Viperidae*, *Elapidae* and *Crotalidae* [37]. Snake venom can be a rich source of pharmacologically active agents. The antibiotic potency of *Crotalus* venom [38] and the lytic factor from the venom of a cobra snake *Hemachatus haemachatus* have been recently reported [39,40]. *Echis carinatus* venom has potent antibacterial effects against *S. aureus* and MRSA, with a minimum inhibitory concentration (MIC) of 80 μg/ml [41]. *Echis pyramidum*, *E. coloratus*, and *Cerastes cerastes gasperettii* venom also potently inhibits bacterial growth. Venom from the black desert cobra (*Walterinnesia aegyptia*) and a phospholipase A_2 (PLA_2) from *Bungarus fasciatus* have greater antibacterial activity against *S. aureus* and MRSA than *Naja haje arabica* [42,43]. Snake venom from *Agkistrodon rhodostoma*, *Bothrops jararaca*, *Bothrops atrox* and *Lachesis muta* is effective against gram-negative and gram-positive bacteria at doses of 1–32 μg/ml. However, *A. rhodostoma/B. atrox* also shows inhibitory potential against *S. epidermidis* and *Enterococcus faecalis* (MIC 4.5 μg/ml) and *B. jararaca* inhibits *S. aureus* (MIC 13 μg/ml) [44]. However, other venoms from scorpions [45,46], wasps [47], and the honey bee (*Apis mellifera*) [48], containing a cationic peptide, mellitin, have strong action against gram-negative bacteria [49,50].

Most conotoxins are disulphide-rich peptides that target receptors of prey [51]. Five different novel conopeptides (Lo6/7a, Lo6/7b, Asi3a, Asi14a and AusB) were tested for antimicrobial activity against 29 gram-positive and 10 gram-negative bacteria and yeast; only Lo6/7a was effective against only one gram-positive bacteria, (*Bacillus megaterium*. Other peptides had no inhibitory effect [52]. Venoms from scorpions, spiders, and terrestrial/sea snakes can also aid in healing various human diseases [35,36].

The natural 'powers' of toxins found in myriad venoms are exquisitely complex, composed of several different types of