Multidrug resistance in bone infection: antimicrobial peptides as a therapeutic tool

Abulfotooh M. Eid[a] and Amira A. Eid[b]

Departments of [a]Orthopedics and Traumatology and [b]Dermatology, Venereology and Andrology, Faculty of Medicine, University of Alexandria, Alexandria, Egypt

Correspondence to Abulfotooh M. Eid, Prof. of Orthopedics Surgery, Departments of Orthopedics and Traumatology, Alexandria University, Alexandria, Egypt
Tel: +01222406037;
e-mail: mohammedghool@yahoo.com

Egypt Orthop J 48:113–116

1110-1148

Egyptian Orthopedic Journal
2013, 48:113–116

Bone infection is a notorious lesion. Despite extensive researches and discoveries in the field of antibiotics and immunity, the old adage 'once osteomyelitis always osteomyelitis' has not lost its relevance [1].

Primary (hematogenous) bone infection is decreasing because of the use of better diagnostic tools and improved healthcare [2]. However, secondary (exogenous) bone infection is increasing because of increased mechanization, road traffic accidents, and implant surgery. Implant surgery and arthroplasty are of particular relevance. Once infection occurs, the acquisition of the implant surface by the host cells or bacteria becomes a crucial 'race for the surface' that determines the clinical outcome [3]. The pathogens soon adhere to the implant surface and form a biofilm. The surface of this film repels antibiotics, reduces antibiotic sensitivity, and increases bacterial virulence. In addition, in the deeper layers of these films, less susceptible, slow-growing bacteria survive. Biofilms of resistant bacteria have been found on gentamicin-releasing polymethyl-methacrylate (PMMA) cement; in one study, 18 out of 20 explanted gentamicin-containing beads showed resistant bacterial strains [4,5].

Superbugs such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *S. aureus*, and vancomycin-resistant *S. epidermidis* have become resistant to last-resort drugs. Unfortunately, the increase in antibiotic-resistant pathogens worldwide coincided with reduced commercial efforts to develop new antibiotics [6]. This itself potentiates the clinical dilemma.

Systemic treatment of bone infection by antibiotics has many limitations because of their side effects. The situation is still worsened by the encapsulation of the infected focus by avascular fibrous tissue and sclerosed bone, which prevents penetration of antibiotics into the infected area [7].

Resort to local treatment of infection, both surgical and medical, has been attempted. Local application of antibiotics has favorable pharmacokinetics, as it produces high drug concentrations at the focus of infection without systemic toxicity [8]. A carrier is needed to make this possible; it contains an antibiotic in a high concentration, from which slow and continuous release of the antibiotic is allowed over a long time, in the expectation that this will eradicate the pathogens. Antibiotics containing PMMA [9] or bead chains [10] have been used. Their disadvantages include the low-grade antibiotic release and the need to remove the beads [8] surgically as they are nondegradable. Furthermore, resistant bacteria may arise on the carrier surface during the later stages of low-level antibiotic release; this necessitates the timely removal of the beads.

Later, biodegradable collagen fleeces were introduced as antibiotic carriers. These can produce high local antibiotic concentrations [11]. They have the advantage of a high level of antibiotic release, obviate the need for removal, and secondary antibiotic release may occur during the degradation phase of the carrier; this could increase the antibiotic efficacy [12].

Therefore, infection is the outcome of the battle between invading pathogens and the immunity of the patient; without adequate immunity, mankind would have disappeared.

In the past decades, a huge armamentarium of antibiotics and chemotherapy has been introduced into that battle. This was preceded by and continued with novel discoveries of antiseptics and antiseptic techniques. The aim was, and still is, not to allow pathogens to settle into human tissues, and if this occurs, to eradicate them. Details of the battle are long and tedious, with genius to and fro maneuvers of the human body and the invaders [13,14]. The human body has immune systems, boosted by proper nourishment and antibiotics. Pathogens have their peculiar and persistent ability to develop resistance through many and varied mechanisms, some of which are known, and yet, many remain unknown [14]. It is a real warfare on a microscopic scale.

Antimicrobial peptides (AMPs) are a new and promising class of antibiotics derived from naturally occurring peptides [15,16]. They have been described as the ancient arm of the human immune system [17,18]. They are enormous and have been found to be genetically and functionally linked with bones [19]. Their main job is to defend the human body and also to offend its invaders [17].

AMPs were first described in 1928, and nisin was the first member. Interest in this group has increased considerably

 DOI: 10.7123/01.EOJ.0000429557.74378.69

over the last 30 years [20]. By the year 2010, more than 1500 AMPs of different origins were reported [21].

AMPs are produced by several species including bacteria, insects, plants, vertebrates, and, in fact, all forms of life. Almost all human tissues and cells typically exposed to microbes can produce AMPs, and they then play a crucial role in human immunity [22].

AMPs have been recognized as ancient evolutionary molecules that have been effectively preserved in mammals [23]. They are expressed on the primary barriers of the organisms, preventing colonization of the host tissues by pathogens [24]. Cutaneous production of AMPs is a primary system for protection and the expression of some AMPs increases in response to infection or injury [21,25–27]. In addition, these peptides are stored in granules within phagocytes, where they aid in the killing of engulfed pathogens [18].

These short, positively charged peptides exert a combined pore-forming and intracellular killing effect on a broad range of microorganisms including resistant bacterial strains, fungi, viruses [1], and certain parasites [28]. An intriguing feature of AMPs is that although microbial and host structures share many features, AMPs achieve specificity by targeting components that host cells lack by exploiting differences between corresponding human and microbial structures and by selectively concentrating polypeptides on microbial surfaces [16]. In addition, some host peptides release or activate latent lytic enzymes (autolysis) of their microbial targets and thereby potentiate their antimicrobial effects. Moreover, specific mechanisms to protect bystander host cells from damage exist. The latter include cell-associated or soluble macromolecules that bind and detoxify AMPs [16].

A unique feature of AMPs is their propensity for inducing antibacterial resistance, which could be of high clinical importance [16]. This may be attributed to the evolutionary difficulty in altering bacterial membrane structure [15,26].

In addition to their antimicrobial effects, AMPs exert many other biological effects [24,28]. These include endotoxin neutralization, chemotactic and immunomodulating activities, induction of angiogenesis and wound repair [27], and antitumoric effects [18]. Their immunomodulating effect results in a higher in-vivo than in-vitro antimicrobial activity by specific activation of signaling cascades in the host immune system [26].

The nomenclature of AMPs has not been standardized. However, there are three major methods to name a newly discovered AMP, namely, peptide property based, peptide source based, and peptide property and source based [19]. The classification of AMPs is still more difficult. They may be classified according to their biological source, biological functions, mechanism of action, on amino acid sequences, or three-dimensional structure [19].

Basically, two very different classes of AMPs exist. The first is represented by gene-encoded, ribosomally synthesized oligopeptides or proteins present in all groups of organisms. The second are nonribosomally synthesized antibiotics produced by bacteria and fungi [17].

In humans, the most documented AMPs are cathelicidins and defensins [20]. A single cathelicidin gene is located on chromosome 3 (CAMP). CAMP encodes an inactive precursor protein referred to as human cationic antimicrobial peptide-18 (hCAP18) with a total length of 170 amino acids [28]. This was first detected in bone marrow cells and keratinocytes of inflamed sites [29,30]. However, many other cells are capable of producing hCAP18, mainly myeloid cells, neutrophils, and mast cells, where cathelicidins remain stored in the granules of these cells [28,31]. Colon enterocytes and epitheloid cells of the urinary and respiratory tracts are another important source of this peptide [26,29].

Defensins are also cationic peptides and are classified into α-, β-, and θ-defensins. They are expressed in neutrophil granules, in paneth cells of the small intestine [32], in the epithelial cells of the female urogenital tract [33], the epithelial cells of the respiratory and urinary tracts [34], and the bone marrow [35].

Vitamin D is a potent inducer of human cathelicidin, hence its role in osteoclast activity [36], as it reduces in-vitro osteoclast differentiation and resorbing activity [18]. Defensins, in contrast, are stimulated by interleukin, tumor necrosis factor α, interferon γ, Gram-negative and Gram-positive bacteria, *Candida albicans*, *Mycobacterium tuberculosis*, and lipoarabinomannan [37,38].

This, in fact, is an oversimplification of a very complicated topic; more details are not within the scope of this paper, which is addressed mainly to orthopedic readers.

The main functions of the AMPs can be classified as follows: (a) direct antimicrobial activity and (b) immunemodulatory properties; these are complementary functions involved in the fundamental role of the AMPs during the control of infectious and inflammatory diseases [26]. The underlying mechanisms are at most conjectural. However, it is generally accepted that cationic AMPs interact by electrostatic forces with the negatively charged phospholipid head groups on the bacterial membrane and cause disruption [26]. They play a role in many infectious diseases, respiratory diseases, bowel diseases, against cancer cells, and wound repair [37,39].

The application of AMPs in musculoskeletal infection is interesting in view of their minimal propensity for inducing microbial resistance [1]. Even after repeated subtherapeutic exposure to AMPs *in vitro*, the occurrence of resistant bacterial strains has remained rare [14]. This is attributed to the large number of natural AMP variants, thousands of which have been identified [26]. Also, the change in the cell membrane potential required to repel AMPs could be difficult for bacteria to induce [40]. However, caution must be exercised against possible future development of resistance to AMPs [41].

The results of preclinical trials using different AMPs particularly human lactoferrin I-II (hLFI-II) and Dhvar-5 in bone infection have been encouraging.

The admixture of gentamicin to PMMA has been mentioned before and has been in use for several decades to prevent or treat orthopedic infections [9]. Unfortunately, gentamicin-resistant staphylococci were found on the surface of the bone cement [3]. This was attributed to the long-term, low-level release of gentamicin following the initial burst release [4,5]. Efforts were directed toward modifying the release kinetics of antibiotics from PMMA bone cement and beads [42]. Prolonging the initial burst release and possibly stopping the low-level sustained release would prevent the selection of resistant bacteria [43,44]. This problem was addressed by the admixture of new antimicrobial agents to PMMA bone cement, aiming at modifying and/or increasing the release of gentamicin from PMMA bone cement [43,45,46]. For this purpose, AMPs were found to be suitable [15].

Dhvar-5, which is an AMP found in human saliva, was used for this purpose. The release of Dhvar-5 from PMMA was ~70% [47] and this was considerably higher than that of gentamicin [9]. In addition, the release of gentamicin was found to increase four-fold by the admixture of Dhvar-5 to osteopal G bone cement [47]. The high release of Dhvar-5 and gentamicin from PMMA bone cement is probably because of increased microporosity of the cement because of the dispersion and subsequent dissolution of Dhvar-5 through the PMMA matrix [47]. Similarly, the AMP hLFI-II, derived from the active domain of human lactoferrin (*N*-terminal amino acid I-II) [48], has been used, with favorable results. Calcium bone cement has been found to be a suitable carrier for hLFI-II [48] and high prolonged in-vitro release of hLFI-II in its biologically active form has been found [49].

In conclusion, the successful in-vitro and in-vivo results of the use of AMPs in prevention and treatment studies of bone infection validate the need for their use in clinical studies of bone and implant infection [1,48]. In addition to antimicrobial action, AMPs have shown diverse biological effects, all of which play a role in the control of infectious and inflammatory diseases [20]. The increased incidence of antibiotic resistance creates an obvious need for new effective and safe treatments. Eventually, AMPs may become useful therapeutic tools as they have been shown to fight not only bacterial but also viral and fungal infections. As their antimicrobial activity is exerted in several ways because of their multifunctional properties, this characteristic makes the development of resistance by microorganisms more difficult [15,16,20,26].

The increased production of AMPs in response to injury or infection is well documented [21,27]. If this could be increased by 'something', it would be a huge asset in the prevention and treatment of infection. Let us hope that the 'something' will be realized in the near future.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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