Review Article

HUMAN DEFENSIN PEPTIDES WITH ANTIMICROBIAL PROPERTIES: AN OVERVIEW

Lalit Lata Jha[1*], Darshika Shah[1], Rajesh KS[1]
1. Department of Pharmaceutics, Parul Institute of Pharmacy, Waghodia, Vadodara, Gujarat, India.
*Corresponding author's E-mail: lalit_lata@hotmail.com

Accepted on: 15-12-2011; **Finalized on:** 20-02-2012.

ABSTRACT

Human defensin peptides with antimicrobial properties are classified under antimicrobial peptides (AMPs). They are produced by neutrophils and epithelial cells and involved in innate immunity through killing of microbial pathogens or neutralizing bacterial toxins and adaptive immunity by serving as chemo attractants and activators of immune cells. They all are 29-35 amino acids peptides, invariably cationic in nature and belongs to β sheet structure with two or three intra molecular disulfide bonds. Many of them attack their target cells by permeabilizing the cell membrane. They can be roughly categorized into three main classes according to their structural differences: the α defensins, β defensins and θ defensins. Most of the known human defensin peptides with antimicrobial functions have been identified and studied during the last 15 years. As a result of these studies, new knowledge has been acquired into biology and biochemistry. It has become evident that these peptides may be developed into useful antimicrobial additives and drugs. The use of some defensin mimetic peptide as replacement for clinical antibiotics is promising. This review focuses on the current status of some of the main types of human defensin AMPs produced by neutrophils and epithelial cells and discusses its mode of action, the novel antimicrobial functions, molecular characterization, new developments, e.g. recombinant DNA production of defensins in bacteria and transgenic plants, novel applications related to these peptides, and future research paradigms.

Keywords: Antimicrobial peptides, Human Defensins, Peptide antibiotics.

INTRODUCTION

Defensins are antimicrobial and cytotoxic peptides that contain 29-35 amino acid residues, including six invariant cysteines whose intramolecular disulfide bonds cyclize and stabilize them in a complexly folded, triple stranded β sheet configuration[1]. It is generated by the proteolytic processing of 93-95 amino acid precursor peptides, they constitute > 5% of the total cellular protein in human and rabbit neutrophils (poly morpho nucleated neutrophils--PMN) and are also produced by rabbit lung macrophages, by mouse and rabbit small intestinal Paneth cells[2,3]. The neutrophilic granulocyte is the most numerous leukocyte in peripheral blood. The development from a multipotent progenitor cell to a mature neutrophil takes place in the bone marrow over a period of 10–14 days[4]. Defensins are widely distributed and abundant 3-4 kDa antimicrobial peptides that are variably cationic and contain six disulfide-paired cysteines. Three structurally distinct peptide families have been identified: 'classical' defensins, wefensins and insect defensins. In many animal species, defensin genes are found in clusters with substantial sequence variability outside the core disulfide-linked cysteines. Defensin peptides have been found in the granules of phagocytes and intestinal Paneth cells, on epithelial surfaces of the intestine and the trachea, and in the hemolymph of insects[5]. They are produced from larger precursors by stepwise, tissue-specific, proteolytic processing, a production resembling that of peptide hormones. Microbes in the phagocytic vacuoles of granulocytes and certain macrophages encounter high concentrations of defensins. Increased transcription of defensin genes and stimulus-dependent release of pre-synthesized defensin-containing cytoplasmic granules contribute to the local antimicrobial response[6]. They are involved in innate immunity through killing microbial pathogens or neutralizing bacterial toxins and in adaptive immunity by serving as chemo attractants and activators of immune cells[7].

MODE OF ACTION OF HUMAN DEFENSINS

The bactericidal machinery of mammalian neutrophils is built up of many components with different chemical properties, involving proteins, peptides and oxygen-dependent radicals. All these components work in synergy, leading to destruction and elimination of ingested microbes. During the eighties, it gradually became clear, that cationic peptides are a part of the oxygen-independent bactericidal effectors in phagocytic cells[8]. They are included as a potent immediate effector molecule in innate immunity and permeate bacterial membranes because of membrane affinity, resulting in lysis of the bacteria. The antibiotic proteins of human neutrophils with three structurally different families that share the capacity to kill bacteria independent of oxygen and evidently, contrary to Metchnikov's conjecture, to do so independent of enzymic action[9]. In another theory they appear to kill mammalian target cells and microorganisms by a common mechanism which involves initial electrostatic interactions with negatively charged target cell surface molecules (likely the head groups of polar membrane lipids), followed by insertion into the cell membranes which they permeabilize, forming voltage-regulated channels[10]. In addition to their antimicrobial and cytotoxic properties, some defensins act as opsonins, while others inhibit protein kinase C, bind specifically to the ACTH receptor and block steroidogenesis or act as selective chemoattractants for monocytes[11].

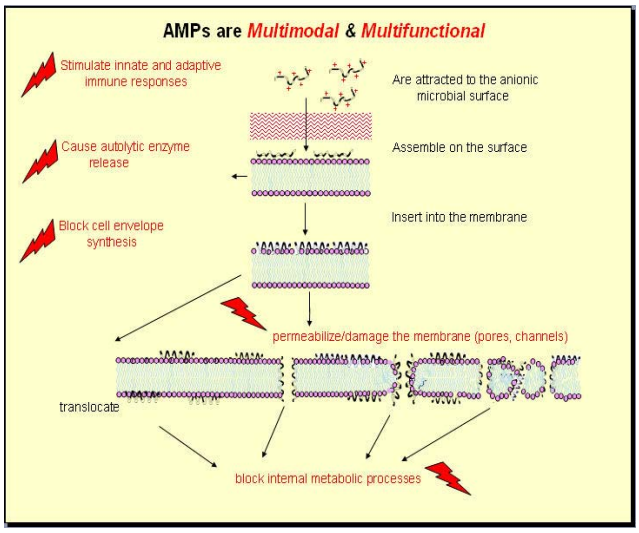

Figure 1: mode of action of antimicrobial peptides taken from www.bbcm.univ.trieste.it/~antimic/ researchAMPs. html[53]

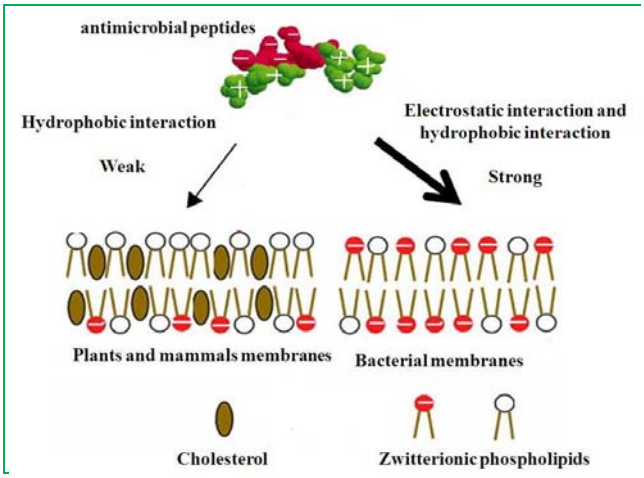

Figure 2: Antimicrobial peptides taken from http://2011.igem.org/Team:St_Andrews/switch[54]

CLASSIFICATION OF HUMAN DEFENSINS AND THEIR NOVEL ANTIMICROBIAL FUNCTIONS

Antimicrobial peptides are small, cationic, amphiphilic peptides of 12–50 amino acids with microbicidal activity against both bacteria and fungi. The eukaryotic antimicrobial peptides may be divided into four distinct groups according to their structural features: cysteine-free α-helices, extended cysteine-free α-helices with a predominance of one or two amino acids, loop structures with one intramolecular disulfide bond, and β-sheet structures which are stabilised by two or three intramolecular disulfide bonds. Mammalian defensins are part of the last-mentioned group. The mammalian defensins can be subdivided into three main classes according to their structural differences: the α- defensins, β-defensins and the recently described θ-defensins. Mammalian α-defensins are predominantly found in neutrophils and in small intestinal Paneth cells. To date, six α defensins have been identified in humans. Four of these, designated Human Neutrophil Peptides (HNP) 1,2,3 and 4, form part of the armoury of neutrophils and they are mainly packed in neutrophil granules, where they participate in systemic innate immunity. The remaining two, Human Defensin (HD) 5 and 6, are expressed in intestinal Paneth cells, and contribute to innate defense of the GI mucosal surface[12]. Mammalian β-defensins have been isolated from both leukocytes and epithelial cells. They are named as human beta-defensins-1 (HBD-1), human beta-defensin-2 (HBD-2), human beta-defensin-3 (HBD-3), and human beta-defensin-4 (HBD-4)[13]. Theta-defensins (cyclic octadecapeptides found in nonhuman primates) have impressive antiviral and antitoxic properties.[17]

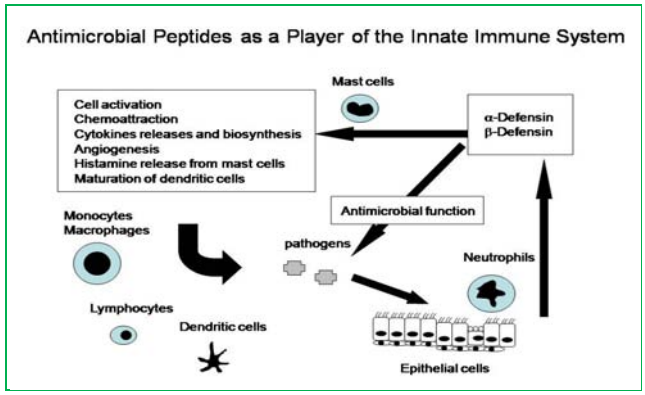

Figure 3: Role of Defensin antimicrobial peptide in innate immune system and as an antimicrobial agent[52]

PRIMARY STRUCTURE OF HUMAN DEFENSINS

The defensins peptides are rich in cystine, arginine, and aromatic residues, but were devoid of free sulfhydryl groups and carbohydrate moieties. They were 29-30 residues in length and identical in sequence in all but their amino terminal residues. The defensins were homologous in sequence to peptides of similar size and biological activity previously purified from rabbit polymorphonuclear leukocytes, but unrelated to other neutrophil proteins of known sequence. 11 amino acid residues of the human defensins, including all six cysteinyl residues, were invariantly conserved in the six rabbit members of this multigene peptide family[48-52].

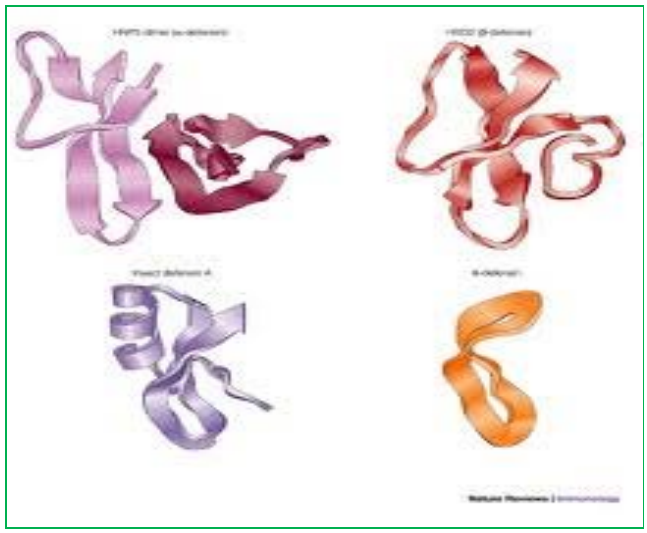

Figure 4: Structure of human defensin[56]

LOCALISATION AND EXPRESSION OF DEFENSINS IN HUMAN CELLS

In the human genome, all known defensin genes cluster to a, 1 Mb region of chromosome 8p22-p23[35]. Studies have shown that Human defensin genes show marked copynumber polymorphism[36]. The microbicidal and cytotoxic peptides made by neutrophils are tissue-specific in expression[37]. The human defensins (particularly HNP1-3) and their mRNAs are found to be localised in cells of normal human bone marrow and peripheral blood[38]. In human peripheral blood, β defensin-1 and -2 genes were transiently transcribed and translated following the induction of lipopolysaccharide or heat-inactivated bacterial cells, whereas α-defensins 1–3 genes were constitutively transcribed, and β defensin-3 gene was not expressed. The inducible expression of β defensin-1 and -2 genes showed interindividual variability[39]. The human defensins are also expressed in normal and damaged peritoneum. This can be shown by immune histochemistry. The reduced expression of some defensins in end stage renal disease is of potential clinical interest against the background of the frequent infective complications seen in peritoneal dialysis[40-42]. Four human epithelial defensins (HD5, HD6, hBD1 and hBD2) is shown to be expressed in the digestive tract. HD5 and HD6 mRNA expression was restricted to the intestine and displayed high interindividual variability. The highest expression levels were observed in jejunum and ileum[43-45].

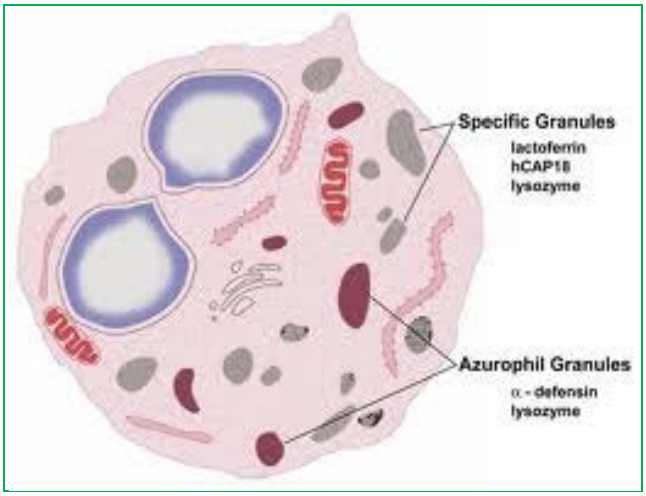

Figure 5: Human neutrophil containing α defensin in Azurophil granules.[55]

BIOSYNTHESIS OF HUMAN DEFENSINS

Human defensins HNP-1 and -3 are broad spectrum antimicrobial peptides that are synthesized by human neutrophils as 94 amino acid (aa) precursors that require proteolytic removal of 64 amino-terminal residues to produce the mature defensins. Recent studies have shown that the early proteolytic processing events include two sequential cleavages, each removing 19 amino-terminal aa residues, that yield 75 aa and 56 aa prodefensins, respectively. The subsequent processing steps that convert these 56 aa prodefensins to mature (30 aa) HNP-1 and HNP-3 are not yet known. Four new defensin precursors in mature normal neutrophils were identified by Sylvia *et al*[46]. The most abundant of these were two 39 aa forms that resulted from the monobasic endoproteolytic cleavage of proHNP-I and proHNP-3. The presence of two proline residues in the vicinity of this newly defined scission site suggested that this cleavage might be "proline-directed." Smaller amounts of the 34 aa and 32 aa prodefensin forms were also found. It remains to be established if these 39, 34 and 32 aa prodefensins are obligate intermediates in the prodefensin processing pathway, or arise from side reactions. In either event, because these prodefensin intermediates accounted for only 0.25% of the total defensin content, proteolytic conversion of 56 aa prodefensins to mature defensins appears to be a highly efficient process[47].

RECOMBINANT DNA PRODUCTION OF DEFENSIN PEPTIDES

Different strategies have been used to clone and express the recombinant defensins in different host systems. The first report of cloning of human defensinHNP-1 was in 1996 which reported that human defensin HNP-I, a small cationic protein, was expressed in E. coli as an insoluble GST fusion protein, and polyclonal antibody raised by immunizing rabbits with this fusion protein did not cross react with human GST and was specific to defensins[28]. Lalitha *et al* have cloned and sequenced a 219 bp coding region of the cDNA of a defensin from Trigonella foenumgraecum, designated as Tfgd2 using primers designed on the basis of a defensin, AlfAFP from Medicago sativa and reverse transcription-PCR. Purified peptide from Escherichia coli expression displayed inhibitory activity against broad-spectrum fungal pathogens, Rhizoctonia solani and Fusarium moniliforme[29]. A vital staining method was also selected as a method for screening and cloning of defensins sequences[30]. Recent studies also used all the peptides, to be expressed as insoluble fusions with the peptide encoded by a portion of E. coli tryptophan operon (trp _LE 1413 polypeptide), were isolated from the inclusion bodies by immobilized metal affinity chromatography (IMAC) and separated from the fusion leader by chemical cleavage[31]. Another approach which was used to clone hBD2 protein is using the vector pET-32a (+) to construct a fusion expression plasmid, pET32–hBD2, which was transformed into E. coli BL21 (DE3) for expression of the fusion protein in soluble form efficiently and to avoid the formation of insoluble inclusion bodies[32]. In one study, multiple copies of the hBD2 gene were linked in tandem, and a number of different Escherichia coli expression vectors were evaluated, including pQE-30, pBV220, pET-28a(+), and pGEX-4T-2. No expression of multiple joined genes was detectable in the pQE-30 expression system, whereas in pBV220 with one or two joined hBD2 genes and in pET-28a(+) with one, two, or four copies, target proteins were expressed at a low level. Only when pGEX-4T-2 was applied as expression plasmid with one or two joined hBD2 genes were target proteins expressed in high

level[33]. Studies have also reported the characterization of the equine α-defensin DEFA (defensin α)[34].

ROLE OF HUMAN DEFENSINS PEPTIDES AS ANTIMICROBIAL, CYTOTOXIC AND CHEMOKINES

Human α defensin peptides have shown to kill majority of gram positive and gram negative bacteria, mycobacteria, T. pallidum, many fungi, and some enveloped viruses[14]. Human β defensin particularly epithelial β defensins are broad-spectrum cationic antimicrobial peptides that also act as chemokines for adaptive immune cells. Human β defensin particularly HBD-1, HBD-2, HBD-3 has microbicidal activity towards the Gram-negative bacteria (Pseudomonas aeruginosa, Escherichia coli) and the yeasts Candida albicans and Malassezia furfur. In addition, HBD-3 also kills Gram-positive bacteria such as Streptococcus pyogenes or Staphylococcus aureus, including multi-resistant S. aureus strains, and even vancomycin-resistant Enterococcus faecium[15]. In contrast to HBD-1 and HBD-2, significant expression of HBD-3 has been demonstrated in non-epithelial tissues, such as leukocytes, heart and skeletal muscle. HBD-4 is expressed in certain epithelia and in neutrophils. Its bactericidal activity against P. aeruginosa is stronger than that of the other known β-defensins[15]. Human Defensins also exert nonspecific cytotoxic activity against a wide range of normal and malignant targets, including cells resistant to TNF α and NK-cytolytic factor[16]. It has been said that high level constitutive expression of defensins may afford protection against HIV-1 and other defensin-sensitive pathogens[17]. Thus multiple properties of defensins contribute to human innate immunity against bacteria, bacterial toxins, and viruses[17]. In addition to antimicrobial response, recent studies have revealed the involvement of defensins in inflammation, immunity and wound repair. The HNP-1 plays an important role in protection against tissue injury during inflammatory conditions by inhibiting the early phase of complement activation[18]. Human defensins also protects skin from microbial invasion[19]. The defensins peptides showed their role in tumour growth, tumour monitoring and cancer treatment[20]. The upregulation of beta-defensins expression in acne vulgaris lesions when compared to controls suggests that beta-defensins may be involved in the pathogenesis of acne vulgaris[21].

DIFFERENT THERAPEUTIC APPLICATIONS OF DEFENSINS

Studies have been reported that the α defensin particularly human neutrophil peptide (HNP)–1 (or defensin 1) can be used as an adjunct to antituberculosis (anti-TB) drugs. The studies have shown that the combination of HNP-1, isoniazid, and rifampicin is active against Mycobacterium tuberculosis H37Rv *in vitro*, *ex vivo*, and *in vivo*, and synergism was observed on the basis of reductions in minimum inhibitory concentrations (MICs) of these agents[22]. Human beta defensin has been shown to contribute to the defence of the intestine against infection by luminal microsporidia spores and may partially determine which parasite species infects the intestine[23]. Some of the artificially synthesised α defensin peptide has also shown useful results against various antimicrobial agents[24]. Some reports also suggest that defensin antimicrobial peptides play an important role in host defence, particularly in the oral cavity where there is constant challenge by microorganisms. Some of the recent findings are that in defensin in addition to their antimicrobial and immunomodulatory effects, particularly, HNP-1–HNP-3 possesses antiviral and toxin neutralizing properties[25]. Some studies also suggest that HNP-1 along with some other agents like SgIII and DMT-1 are implicated in cell-mediated LDL oxidation for pathogenesis of atherosclerosis[26]. Different studies have also suggested that antimicrobial defensin (HD 5 and HD 6) peptides are important in protection of the host against microbial invasion in states of intestinal inflammation[27].

CONCLUSION

Antimicrobial defensin peptides can be a successful drug for natural antibiotic therapy particularly for the resistant bacterial species for which synthetic antibiotic are not effective. The defensin can be produced either synthetically or by recombinant DNA technology. An effective cloning and expression technique will lead to biologically active defensin production in the lab which is cost effective and cheap as compared to production of this peptide by synthetic route. Further a suitable route of administration can be designed to deliver stable defensin peptide in body.

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