Clinical development of peptide antibiotics

Discovery, development and initiation of therapeutic use of antibiotics, e.g. penicillin, have had a tremendous impact on human life expectancy, creating a safety net and leading to a rapid decline in the rate of discovery of new antimicrobials, e.g. only three new chemical classes of antibacterials have entered the market in the past 40 years – i.e: lipopeptides, oxazolidinones and streptogramins, all targeting Gram-positive infections – in parallel with a rapid increase in the incidence of multi-drug resistant pathogens. Fueled by an increasing understanding of the intrinsically complex and conserved immune signaling system, i.e., identification of specific pathogen recognition receptors, such as Toll-like receptors, and intracellular sensors of microbial components such as the Nod-like receptors and retinoic-acid inducible gene I like receptors (1). Followed by recently launched publicly-available bioinformatics resources like, Innate DB (www.innatedb.com) (2) and IIDB (http://db.systemsbiology.net/IIDB) (3), which are innate immunity specific databases, able to interrogate the immune pathways in response to pathogens and drug candidates.

Academic researchers have increased their commitment on tailoring novel strategies for microbial intervention, especially targeting the host’s own immediate defence system, innate immunity. Several exciting potential targets are currently being pursued by different strategies, like antibodies, biologicals – i.e. proteins and peptides – and small molecule agonists and antagonists; their success has been reviewed elsewhere (4-9).

Another very promising drug class is so called host defence peptides– HDPs; or cationic antimicrobial peptides (AMPs) when they have direct antimicrobial activity – and synthetic derivative thereof. The therapeutic use and clinical potential of HDPs/AMPs and peptide drugs in general, are discussed in brief in this review.

HOST DEFENCE PEPTIDES

Host defence involves a complex interplay between innate (germline encoded) and adaptive (antigen specific) immunity, and host defence peptides have been demonstrated to be a key player in the orchestration of innate immune and inflammatory responses of mammals, amphibians and insects (10-12), thus bridging these two immune defence strategies. The pivotal role as signature molecules of host defence render AMPs/HDPs ubiquitous at moderate to high concentrations, present in virtually all species of life, with more than 1000 natural occurring peptides been described to date. The majority of these are described in databases for eukaryotic host defence peptides: e.g. the site at the

Figure 1- Structural classes of antimicrobial peptides:
(A) mixed structure of Plectasin, a defensin-like molecule (PDB code 1ZFU) (48); (B) β−helical lactoferricin (PDB code 1LFC) (3); (C) α−helical human cathelicidin LL-37 (PDB code 2K6O) (75); (D) extended indolicidin (PDB code 1G89) (76).

The figures have been prepared with use of the graphic program MolMol 2K.1 (77)

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PEPTIDES
These peptides are generally short (12 to 50 residues), with a net positive charge (+2 to 9) due to excess basic arginine/lysine residues, and contain up to 50% hydrophobic amino acids. They are typically sorted into four structural classes based on their amphiphilic conformations; namely β-structures with two to four β-strands, amphipathic α-helices, loop and extended structures (Figure 1). Most of these peptides carry a broad spectrum of activities, i.e. direct antibacterial, antifungal, antiviral and antiparasitic activity (15), activities which are strongly antagonized by physiological salt concentrations i.e. 100 mM monovalent- and 2 mM divalent-cations (16). In addition to these direct antimicrobial properties, it has also been recognized that many of these peptides modulate host cell immune responses,
though recruitment and activation and/or modulation of effectors of the innate immune system (12,17). HDPs/AMPs has often served as a template for design of new peptide antibiotics giving researchers insight into important systemic effects of peptide antibiotics in general.

**HOST DEFENCE PEPTIDES MODE OF ACTION**

Classically cationic host defence peptides were investigated for their direct antimicrobial activity. Though the peptides usually demonstrated lower potency than conventional antibiotics, they possessed the ability to kill multidrug resistant bacteria through extremely rapid mechanisms, targeting and inhibiting multiple bacterial targets (18). Initially the peptides were believed to act only by perforating the bacterial membranes through, e.g. "aggregate" (19), "toroidal pore" (20-23), "barrel-stave" (24), or "carpet" mechanistic models (25). However, later studies have also demonstrated the ability of many peptides to translocate across the bacterial cytoplasmic membrane (26) targeting DNA/RNA synthesis (27-30), protein synthesis (28,29,31,32), enzymatic activities (33,34) and cell wall regeneration (35) amongst others. Supplementing this direct antimicrobial activity, there is strong evidence that a broad range of cationic peptides can stimulate the host immune system, promoting pathogen clearance (Figure 2). Thus the terms AMPs and HDPs are to some extent interchangeable, though the term HDPs is usually used for natural peptides with known immunomodulatory properties, while AMPs only are known for their direct antimicrobial effects.

However many natural peptides possess both features, and have been implicated as key players in innate immunity. For example, patients suffering from Crohn's disease, experiencing chronic intestinal inflammation (36), have also been shown to have deficient secretion of β-defensins 2 and 3 (37,38) and the classical α-helical cathelicidin LL-37 (39). Very similar to this neutrophile levels of LL-37 and α-defensin-1, -2 and -3 are significantly reduced in patients suffering from morbus Kostmann disease, a sever gum inflammation (40). Similarly, researchers have been able to knock out CRAMP in mice, the peptide homologue to human LL-37 (41,42). These mice then demonstrate significant reduction of cytokine production as well as T-cell dysfunction (43). Histatin 3/5 demonstrating antifungal properties against Candida albicans when used as a mouth rinse at very low concentrations (0,15%), in immunocompromised patients (Phase Iib study). CENZ-002 is another shorter 8-mer peptide modified from α-melanocyte-stimulating hormone, which is investigated by Zengen. This peptide has also demonstrated antifungal potential in Phase Iib.

A handful of peptides are being developed for their antiviral properties. A di-peptide (IM862) has been isolated from the calf thymic peptide complex Thymalin (57). Research has indicated that IM862 is able to stimulate the production of immune cells and to normalize their numerical relationship under immune deficiency conditions, thus demonstrating efficacy against both viral and tumor assaults (58). A sodium carboxylated salt of IM862 (Oqylufadine disodium) are currently being put through Phase Ila clinical trials by Implicit Biosciences investigating efficacy of this for defeating hepatitis C virus infections (Table I). A synthetic derivative of IM862 named SCV-07 has also proven immune stimulating properties, through its effects on Tp1 cells, which are essential for clearance of viral infections. Phase II trials in Russia have also demonstrated that SCV-07 can be used in combination with standard chemotherapy to fight tuberculosis (59). An ongoing consecutive Phase Ila clinical trial aims at evaluating its efficacy on reduction of hepatitis C viral load as well as on other measures of immunomodulating response (60) (Table I). SCV-07 has also recently demonstrated significant reduction of recurrent lesions, when administered orally, but not subcutaneously in a guinea pig model of recurrent genital herpes simplex virus 2 (61). Another peptide originally isolated from calf thymus is a 28-mer polypeptide named Thymosin α1 (ZADARIN®) (62). It is able to enhance Tp1 cytokine production as well as T-cell differentiation and maturation to augment a
specific T lymphocyte function like the Th responses involved in antiviral defence (63). The peptide is currently being synthesized on solid phase by SciClone Pharmaceuticals International and it has been approved in more than 30 countries for treatment of hepatitis B and C virus infections and some cancers. However, despite approval, clinical trials comparing ZADAXIN® to other antiviral treatments have been scarce and demonstrated inconclusive (64-67) or negative results (68). Interestingly, human trial studies have also demonstrated improved survival for patients infected with carbapenem-resistant bacteria, when treated with ZADAXIN® in combination with human milk protein lactoferrin, an well investigated protein known to carry both antimicrobial as well as immune modulatory properties (70,71). AM pharma is developing hLF1-11 for protection and prevention of infections during allogeneic stem cell transplantation. The direct antimicrobial Omiganan (described earlier) has also demonstrated an ability to suppress acute acne and Rosacea in Phase II clinical trials, illustrating the extremely complex and multifaceted actions of these peptide derivatives. Another immune modulating peptide is a semi-synthetic D-amino acid 10-mer, RDP58, derived from the heavy chain of HLA class 1 molecule (72). Genzyme is currently preparing RDP58 for Phase II trials, were protection and/or dampening of inflammation will be investigated in patients suffering from inflammatory bowel diseases. A newcomer in

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Table I: Selected peptides in clinical trials or developmental stages

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sequence</th>
<th>Description / Status / Results</th>
<th>Company &amp; Reference</th>
</tr>
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<tbody>
<tr>
<td>PTX002</td>
<td>SQIDILNSMKLFRKQ AKNKVIKVLNGRELS LD-CD-COOH</td>
<td>Discovery phase: 33-mer peptide with two distinct β-sheet folds and a 12-mer peptide derivative thereof. Has demonstrated broad-spectrum antimicrobial and antiendotoxin neutralizing activity in both in vitro and in vivo experiments.</td>
<td>PepTex (St. Paul, MN, USA) peptx.com (78, 79)</td>
</tr>
<tr>
<td>PTX005 / SC4</td>
<td>KLFKRLHKWIKI-COOH (Plectasin)</td>
<td>Preclinical: A variant of plectasin which has demonstrated potent Gram-positive effect in systemic pneumococcal and streptococcal infections.</td>
<td>Novozymes AS (Bagsværd, Denmark) novozymes.com</td>
</tr>
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this group is a peptide (IMX942) derived from innate defence regulator-1 (IDR-1), an anti-infective peptide that selectively modulates the innate immune response (73). IMX942 is being developed by Inimex Pharmaceuticals and is currently undergoing Phase I safety testing in healthy volunteers with very promising results, and preparations are already underway to begin Phase II clinical testing, evaluating efficacy in targeting pneumonia, surgical site infections and chemotherapy induced neutropenia in the first quarter of 2010 (74).

CONCLUSION

We are entering the “post-antibiotic era”; or at best antibiotics are progressively demonstrating decreased efficacy. The overwhelming interest in antimicrobial peptide research that were experienced 20 years ago, slowly died off as at first generation antimicrobial peptide drug candidates failed in late stage clinical trials. However, much of the enthusiasm in this field is now back and several very interesting and diverse peptide drug candidates are currently being followed through clinical testing. The complexity of the innate immune system clearly dictates the numerous potential novel targets for such peptide drugs. The peptides flexibility and attraction for different targets followed by the intertwined mode of actions of these peptides, underscore the complexity of this strategy but also the potential for success. Development of peptide drugs are now more than ever an innovative and very interesting strategy that if pursued with research and adaptation of new technology platforms one day may carry significant fruits.

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