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Standard Review

Therapeutic potential of antimicrobial peptides from insects

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The first antimicrobial peptides were isolated from the cecropia moth *Hyalophora cecropia* in 1980. Since then a plethora of antimicrobial peptides have been isolated from other arthropods, invertebrates and chordates. With the emergence of antibiotic resistant bacterial pathogens and the promising activity of these peptides, attempts are being made to use these peptides as new antimicrobial agents. Other researchers are interested in using these peptides to improve the resistance of crops and livestock to infections, while another line of research is interested in using these peptides to control vector borne diseases. Despite the promising antibacterial, antiviral, anti-protozoan and anti-tumor activity of these peptides, relatively few peptides have made it to clinical trials. Problems associated with the development of these peptides into effective antimicrobial agents include their higher cost, proteolysis or decreased activity in physiological environments and mass production. This review will focus specifically on the development of insect antimicrobial peptides into useful chemotherapeutic agents.

Key words: Insect, antimicrobial peptide, drug discovery.

INTRODUCTION

Interest in antimicrobial peptides began in 1980 when cecropin was isolated from the cecropia moth *Hyalophora cecropia.* This was 50 years after the initial observations in the 1920s that insects released a bacteriolytic substance into their blood when challenged with bacteria. Since the isolation of cecropin there has been interest in the use of these antimicrobial peptides in therapeutic, biocontrol and agricultural applications. In total there are about 559 antimicrobial peptides identified to date, isolated from plants, vertebrates and invertebrates. Of these peptides the majority are bactericidal, a significant amount are antifungal while only a small number are anti-viral or act against cancer cells (Wang and Wang, 2004).

Most antimicrobial peptides tend to be highly basic, which facilitates their interaction with the microbial cell membrane (Lauth et al., 1998). All of these antimicrobial peptides are synthesised as precursor peptides that can be up to five times larger than the active peptide (Trenczek, 1997).

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Antimicrobial peptides can be divided into classes based on their amino acid sequence and structural characteristics, as follows:

1) The linear amphipathic α helix forming peptides (Bulet et al., 2004) (Table 1, Figure 1A).
2) The cystine rich or cyclic antimicrobial peptides (Table 2, Figure 1 B-F).
3) The lysozymes.
4) The proline rich peptides (Otvos, 2002) (Table 3).
5) The glycine rich peptides (Rees et al., 1997) (Table 3).

Classes 4 and 5 apply specifically to insect antimicrobial peptides and may be combined into a class of peptides rich in one or more amino acids.

This review will focus on the development of insect antimicrobial peptides into useful therapeutic products and their application to agriculture and disease prevention. This will be followed by a description of the

Table 1. Amphipathic linear antimicrobial peptides (AMPs) from different organisms. Linear amphipathic antimicrobial peptides isolated from plants and animals. The table includes mainly arthropod antimicrobial peptides, and some vertebrate and plant peptides.

Sources	Name	Size (amino acids)	Structure	Activity	Commercialised or not	References
Arthropods						
Lepidoptera	Cecropin	31-39	α helix hinge α helix	Gram negative, Gram positive	No	Steiner et al. (1981)
Diptera	Cecropin	31-39	α helix hinge α helix	Gram negative, Gram positive	No	Okada and Natori (1983)
Dipteran (mosquito)	Cecropin	31-39	α helix hinge α helix	Gram negative, Gram positive, Protozoa	No	Lowenberger et al. (1999)
Synthetic (cecropin analogue)	Shiva-3	35	α helix hinge α helix	Gram negative, Gram positive, Protozoa	No	Jaynes et al. (1988)
Synthetic cecropin analogue	D2A21	23	α helix hinge α helix	Bacteria, Virus, Tumor	Yes	Chalekson et al. (2003)
Oxyopes kitabensis	Oxypinin	48	α helix	Bacteria, Hemolytic, Fungi	No	Corzo et al. (2002)
Parabuthus schlechteri	Parabutoporin	40–50	α helix	Bacteria, Hemolytic, Fungi	No	Moerman et al. (2002)
Opistophtalmus carinatus	Opistoporin	40–50	α helix	Bacteria, Hemolytic, Fungi	No	Moerman et al. (2002)
Lycosa carolinensis	Lycotoxins	25-27	α helix	Gram negative bacteria, Yeast	No	Yan and Adams (1998)
Cupiennius salei	Cupiennin	35	α helix hinge, α helix	Bacteria	No	Kuhn-Nentwig et al. (2002) and Pukala et al. (2007)
Pachycondyla goelidii	Ponericin G	30	α helix	Bacteria, Insecticidal, Hemolytic	No	Bulet et al. (2004) and Orivel et al. (2001)
Stomoxys calcitrans	Stomoxyn	42	α hinge α hinge α	Bacteria, Fungi, Yeast, Protozoa	No	Boulanger et al. (2002b)
Psuedocanthotermes spiniger	Spinigerin	25	α helix hinge α helix	Gram negative, Gram positive, Fungi	No	Lamberty et al. (2001a)
Bombyx mori	Moricin	42	α helix	Gram positive, Gram negative	No	Hara and Yamakawa (1995)
D. melanogaster	Andropin	34	α helix hinge α helix	Gram positive	No	Samakovlis et al. (1991)
Ceratitis capitata	Cerratotoxin	29	α helix	Gram positive, Gram negative, Hemolytic	No	Marchini et al. (1993)
Apis mellifera	Mellitin	26	αα	Gram positive, Gram negative, Hemolytic	No	Anderson et al. (1980)
Synthetic (mellitin analogue)	Hecate	23	α helix hinge α helix	Bacteria, Virus, Tumor	No	Arrowood et al. (1991)
Agelaia pallipes pallipes	Protonectin	12	α helix	Bacteria	No	Mendes et al. (2004)
Vertebrates						
Sus scrofa	Cecropin P1	31	α helix hinge α helix	Bacteria	No	Lee et al. (1989)
Styela clava	Styelin	32	α helix	Bacteria, Heolytic	No	Zhao et al. (1997)
	Clavanin	23	α helix histidine rich	Bacteria	No	Lee et al. (1997)
Xenopus laevis	Magainin-2	23	α helix	Bacteria, Fungi	No	Zasloff (1987)
Sythetic Magainin	Pexiganan	22-	Magainin analogue	Bacteria	Passed Phase III trial	Jacob and Zasloff (1994)
Rana temporaria	Temporin	10-13	α helix	Bacteria, Fungi, Hemolytic	No	Simmaco et al. (1996)
Ltioria species	Caerin	25	αα	Bacteria	No	Steinborner et al. (1997)
Rana rugosa	Gaegurin	37	Helix kink helix	Bacteria	No	Park et al. (1995)
Homo sapiens	Ovispirin-1	18	α helix	Bacteria, Fungi, Hemolytic	No	Sawai et al. (2002)
Synthetic ovispirin	Novispirin G-10		α helix	Bacteria, Fungi	No	Sawai et al. (2002)
Other						
Entamoeba histolytica	Amoebapore	77	αααα	Bacteria, Fungi	No	Lynch et al. (1982)

problems associated with the development of antimicrobial peptides as therapeutic treatments.

MODES OF ACTION

Antimicrobial peptides generally act in two ways. Firstly, they act by destabilizing the cell membrane and secondly by entering the cell and interacting with specific targets. However, before these peptides can act on the bacterial membrane, they must first pass the bacterial cell wall. A possible way this could be accomplished is the self promoted uptake model. This model relies on the displacement of polyanionic cations that bridge the LPS molecules. This disrupts the outer wall through which peptides pass (Shai et al., 2001).

Those peptides which destabilize the cell membrane are thought to act by one of three models. The first model is the barrel stave model, where peptide monomers associate and form a pore in the membrane made up of bundles of helices (Shai et al., 2001). The second model, the carpet model, involves peptides gathering on the membrane surface and forming toroidal pores which disrupt membrane structure (Huang, 2000). The final model is the toroidal pore model which involves the peptides binding to the surface of the membrane and causing a thinning of the membrane as the peptides associate with lipid headgroups (Huang, 2000).

APPLICATIONS OF ANTIMICROBIAL PEPTIDES

Since the discovery of penicillin antibiotics have been widely used to combat many previously fatal infectious diseases. However, their continued widespread and excessive use has led to the emergence of many antibiotic resistant strains. Much of the current interest in antimicrobial peptides is a direct result of these antibiotic resistant pathogens and the consequent need for new antibiotics. Many think that antimicrobial peptides could be the new generation of antibiotics.

There are many ways in which antimicrobial peptides may play a role as antimicrobials or therapeutic agents. Firstly, they can be used as stand alone antimicrobial agents, such as conventional antibiotics. Secondly, they can be used in conjunction with other antimicrobial agents to increase the efficiency of antimicrobial activity. Thirdly, they can be used to enhance the patient's own innate immune system. Finally, they can be used to neutralize endotoxins resulting from septic shock (Gordon et al., 2005).

Stand alone antimicrobial agents

Many bacterial pathogens that are becoming increasingly resistant to current antibiotics have been found to be sensitive to antimicrobial peptides isolated from insects. For example it has been found that strains of the pathogenic *Staphylococcus aureus* that are resistant to antibiotics such as methicillin, are sensitive to the defensin isolated from the beetle *Allomyrina dichotoma* (Yamada et al., 2005). A cecropin analogue peptide, D2A21, was shown to be more successful in the treatment of infected wounds than standard treatments, with 100% of rats with infected wounds surviving compared to a 50% survival rate in the control (Chalekson et al., 2003). There are also peptides isolated from chordates with similar activity. Halocidin, a peptide isolated from a tunicate, was active against resistant strains of *S. aureus* and *Pseudomonas aerginosa.* Halocidin is a heterodimer consisting of an 18 residue domain linked to a 15 residue domain via a disulfide bond. It was found that a synthetic 18 residue heterodimer was more active than the natural peptide (Jang et al., 2002).

Antimicrobial peptides have been extensively investigated for their use as ophthalmic and dental antimicrobials. In ophthalmic applications the peptide can be applied directly to the infected site in the form of eye drops and the amount of active peptide can easily be increased by additional dosing (Gordon et al., 2005). Despite these advantages the only sign of antimicrobial peptides having a promising application in ophthalmology applications, is in the disinfection of contact lenses. The increased use of contact lenses has also led to an increase in contact lens associated cornea infections (Sousa et al., 1996). Cecropin analogues Shiva-11, D_5C as well as hecate were all tested for their abilities to disinfect contact lenses as well as contact lens solutions. D_5C was able to exponentially increase the ability of existing contact lens sterilizing solutions to sterilise contact lenses (Sousa et al., 1996). Shiva 11 and hecate were both able to kill bacterial isolates from infected contact lenses (Gordon et al., 2005).

Antimicrobial peptides isolated from insects have been shown to have the ability to prevent mortality in mice that have been infected by influenza virus. These peptides, named alloferons, were isolated from the blow fly *Calliphora vicina*. Alloferon shares some structural elements to the influenza virus hemaglutinin protein. This may mean that alloferon is able to either interfere with viral assembly, or viral attachment to the cell (Chernysh et al., 2002). A highly successful mellitin derivative named hecate was produced by altering the charge distribution of mellitin, while at the same time retaining its three dimensional structure (Baghain et al., 1997). Hecate demonstrated antiviral activity against *herpes simplex virus-1*. At relatively low concentrations, hecate reduced plaque formation. However, it did not interfere with the virus's ability to synthesize proteins. Furthermore, it was observed that Hecate was able to prevent (HSV-1)-induced cell fusion and virus spread, with no cytotoxic effects (Baghain et al., 1997).

Both cecropin and mellitin were able to inhibit the production of HIV-1 in infected cells. These peptides

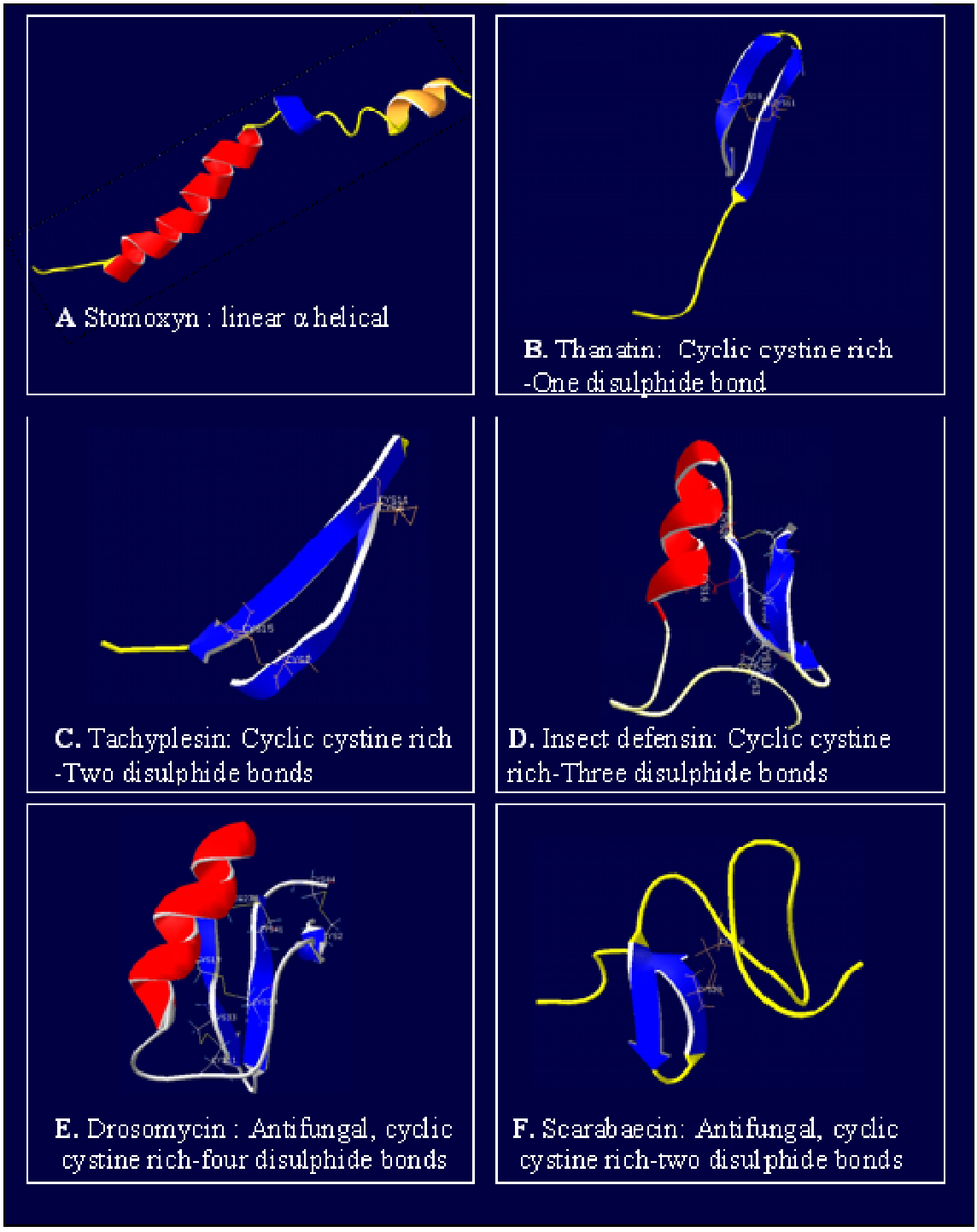

Figure 1. Structural representations of some arthropod anti-microbial peptides representing the different classes. Structural information was obtained from the Protein Data Bank (Berman et al., 2000). (A) The linear α helical peptide stomoxyn (Landon et al., 2006) isolated from the dipteran *Stomoxys calcitrans.* (B) Thanatin (Mandard et al., 1998), isolated from the hemipteran *Podisus maculiventris*, is the only insect cyclic cystine rich peptide with only one disulphide bond. (C) Cyclic cysteine rich peptide containing two disulfide bonds tachyplesin (Laederach et al., 2002), isolated from the horseshoe crab *Tachyplesus tridentatus.* (D) The insect defensin isolated from *Phormia terranovae* (Cornet et al., 1995) (E) The antifungal peptide drosomycin (Landon et al., 1997) is a cyclic cystine rich peptide containing 4 disulphide bonds. (F) Another antifungal peptide scarabeacin (Hemmi et al., 2003) was isolated from the rhinoceros beetle *Orychtes rhinoceros*. Although this peptide is a cyclic cystine rich peptide with two disulfide bonds, its structure is very different to that of tachyplesin.

Table 2. Cyclic cystine rich antimicrobial peptides (AMPs) from different organisms (plants and animals).

Sources	Name	Size (amino acids)	Structure	Activity	Commercialised or not	References
			Invertebrates			
One disulphide bond						
Podisus maculiventris	Thanatin	21	Anti parallel β sheet with a tail	Gram positive, Gram negative Fungus	No	Fehlbaum et al. (1996)
Mymecia pilosula	Pilosulin	24-36	β sheet	Bacteria	No	Inagaki et al. (2004)
Two disulfide bonds						
Oryctes rhinoceros	Scarabeacin	38-40	Two stranded antiparrallel β-sheet after a helical turn	Fungi	No	Tomie et al. (2003)
Acanthoscurria gomesiana	Gomesin	18	Anti parallel β sheet	Bacteria, Fungi, Yeast, Hemolytic, Eukaryotic, Parasites	No	Mandard et al. (2002)
Androctonus australis	Androctonin	25	Anti parallel β sheet	Yeasts, Bacteria Fungi	No	Bulet et al. (2004)
Limulus polyphemus	polyphemusin	18	Anti parallel β sheet	Yeasts, Bacteria, Fungi	No	Bulet (2004: 162)
Tachyplesus tridentatus	tachyplesin	17	Anti parallel β sheet	Yeasts, Bacteria, Fungi, Hemolytic, Anti viral	No	Kawano (1990: 2)
Three disulfide bonds						
Acrocinus longimanus	Alo-3	36	Knottin type fold	Yeast	No	Barbault et al. (2003)
Tachypleus tridentatus	tachystatin					Osaki et al. (1999)
Mytillus edulis	Defensin	38-43	Disulfide-stabilised αβmotif	Gram positive Gram negative	No	Charlet et al. (1996) and Mitta et al. (1999)
Dipteran	Defensin	38-43	Disulfide-stabilised αβmotif	Gram positive	No	Chalk et al. (1995), Matsuyama and Natori (1988a), Lehane et al. (1997), Boulanger et al. (2002a) and Lauth et al. (1998)
Hemipteran	Defensin	42-43	Disulfide-stabilised αβmotif	Gram positive	No	Cociancich et al. (1994), Chernysh et al., 1996) Lopez et al., 2003)

Table 2. Contd.

Hymenoptera	Defensin	38-43	Disulfide-stabilised αβmotif	Gram positive	No	Rees et al. (1997)
Arachnida	Defensin	38-43	Disulfide-stabilised αβmotif	Gram Positive, Gram negative	No	Ehret-Sabatier et al. (1996), Johns and Sonenshine (2001) and Cocianich et al. (1993)
Coleopteran	Defensin	43	Disulfide-stabilised αβmotif	Gram Positive, Gram negative	No	Yamaauchi (2001) and Moon et al. (1994)
Aeschna cyanea	Defensin	38		Gram positive, Gram negative	No	Bulet et al. (1992)
A. gambiae	Defensin	38-43	Disulfide-stabilised αβmotif	Gram positive	No	Richman et al. (1997)
Apis mellifera	Royalisin	51	Disulfide-stabilised αβmotif	Gram positive	No	Fujiwara et al. (1990)
Lepidoptera	Defensin	32-36		Gram positive	No	Seitz et al. (2003), Volkoff et al. (2002) and Mandrioli et al. (2003)
Pseudacanthotermes spniger	Termicin	36	áââ cysteine stabilized motif	Fungi	No	Lamberty et al. (2001a)
Heliothis virescens	Heliomicin	44	βαββ	Fungi, Yeast	No	Lamberty et al. (1999)
Synthetic Heliomicin analogue				Fungi	Pre-clinical trials	
Ascaris suum	ASABF	71		Bacteria	No	Pillai et al. (2003)
Drosophila melanogaster	Drosomycin	44	βαββ	Fungi	No	Fehlbaum et al. (1994)
Mytillus edulis	Mytillin			Gram positive, Gram negative	No	Charlet et al. (1996) and Mitta et al. (1999)
5 disulfide bonds						
Tachypleus tridentatus	Tachycitin	73	β sheet	Gram-negative, Gram positive Fungi	No	Suetake et al. (2000)
			Vertebrate			
One disulphide bond						
Halocynthia aurantium	Halocidin	32	Two subunits connected by a single disuphide	Bacteria	No	Jang et al. (2002)
Rana brevipoda	Brevinin	24	Hydrophobic region, a proline hinge C-terminal loop region	Bacteria	No	Morikawa et al. (1992)
Rana esculenta	Esculentin	46		Bacteria	No	Simmaco et al. (1993)
Rana catesbeiana	Ranalexin	20	α-helical	Bacteria	No	Clark et al. (1994)

Table 2. Contd.

Two disulfide bonds						
Sus scrofa	Protegrin I	17	β sheet	Bacteria, Viruses, Fungi	No	Storici and Zanetti (1993)
Synthetic protegrin	Iseganan			Bacteria, Fungi	Failed Phase III trial	Toney (2002)
Three disulfide bonds						
Gallus gallus	Gallinacin-1	36-39			No	Harwig et al. (1994)
Mus musculus	Cryptidin	70		Bacteria	No	Ouellette et al. (1989)
			Plants			
Phytolacca americana	PAFP-S	38	Knottin-type fold	Fungi	No	Shao et al. (1999)
Triticum turgidum	γ-1-P thionin			Fungi, Bacteria, Tumor cells	No	Carrasco et al. (1981)
Chassalia parviflora	Circulin A	30	Knottin-type fold	HIV	No	Fujikawa et al. (1965) and Daly et al. (1999)
			Fungi			
Pseudoplectania nigrella	Plectasin	40	3 disulfide Coil αhelix coil	Bacteria	Yes	Fungi

achieve this by decreasing both HIV-1 transcription and the number of viral gene products (Wachinger et al., 1998)from the beetle *Allomyrina dichotoma* were found to protect mice from endotoxic shock by acting as anti-inflammatory agents. The peptides were found to accomplish this by inhibiting the production of tumor necrosis factor α (Koyama et al., 2006). The authors suggested a possible mechanism underlying the peptides' ability to block TNF-α production. This involves the peptide preventing LPS from binding to LPS receptors on the surface of macrophages (Koyama et al., 2006). Moreover, the small alloferon peptides isolated from C. vicina were able to stimulate interferon production in mice as well as stimulating mouse spleen lymphocyte cytotoxicity (Chernysh et al., 2002). The horseshoe crab peptide Tachyplesin III was also able to efficiently kill *P. aeruginosa*– a multidrug resistant pathogen. This effect was significantly enhanced when conventional antibiotics were used in conjunction with tachyplesin (Cirioni et al., 2007). Like the beetle defensins, tachyplesin was also able to protect the mice from endotoxic shock following bacterial lysis (Cirioni et al., 2007). The highly effective hemipteran peptide thanatin was found to be effective against multidrug resistant isolates of *Enterobacter aerogenes and Klebsiella pneumoniae*. These strains show increased resistance to antibiotics due to increased altered membrane permeability, which allows them to expel antibiotics regardless of structure. Like tachyplesin III, thanatin also restored the antibiotic susceptibility of these resistant isolates. This must be achieved by making the membrane of the bacteria more porous to antibiotics or interfering with the bacteria's ability to expel specific character of the cell membrane. Comparison of bacteria and tumor cell membranes point to the common characteristic that both are negatively charged (Leushner and Hansel, 2004). This is due to tumor cell membranes containing a small amount of negative phosphatidylserine, making them 3-9% more negative than normal cells (Zwaal and Schroit, 1997).

Mellitin derivatives have been produced by changing a few L-amino acids with D-enantiomers.

Table 3. AMPs rich in particular amino acids from different organisms.

Sources	Name	Size (amino acids)	Structure	Activity	Commercialised or not	References
			Invertebrates			
Drosophila melanogaster	Drosocin	19	Proline rich O-glycosylated	Gram negative	No	Bulet et al. (1993)
Phormia terranovae and other diptera	Diptericin	100-110	O glycosylated glycine rich domain and a proline rich domain	Gram-negative bacteria	No	Dimarcq et al. (1988)
Palomena prasina	Metalnikowin	15-16	Proline rich non-glycosylated	Fungi	No	Chernysh et al. (1996)
Hymenoptera	Apideacin	19	Proline rich non-glycosylated	Gram negative	No	Casteels et al. (1989)
Hymenoptera	Abeacin	39	Proline rich non-glycosylated	Bacteria	No	Casteels et al. (1990)
Bombyx mori Trichoplusia ni	Lebocin	179 133	Proline rich O-glycosylated	Bacteria	No	Hara and Yamakawa (1995), and Liu et al. (2000)
Drosophila melanogaster	Metchnikowin	28	Proline rich O-glycosylated		No	Levashina (1998: 75)
Pyrrhocoris apterus	Pyrochoricin	20	Proline rich O-glycosylated reverse turns at both the C and N termini	Gram-positive bacteria	No	Cociancich et al. (1994)
Myrmecia gulosa	Formeacin	16	Proline rich O-glycosylated	Gram negative	No	Mackintosh et al. (1998b)
Hyalophora cecropia and other lepidoptera	Attacin	214-224	Random loops and B sheets	Gram-negative	No	Hultmark et al. (1983)
Sarcophaga peregrina and other diptera	Sarcotoxin	63	Random loops and B sheets	Gram-negative	No	Ando et al. (1987)
Pyrrhocoris apterus	Hemiptericin	133	Glycine rich Equally large number of positively and negatively charged residues	Gram negative bacteria	No	Cociancich et al. (1994)
Zophopas atratus	Coleoptericin	74	Glycine rich	Gram-negative bacteria	No	Bulet et al. (1991)
Holotrichia diomphalia..	Holotricin	72	High glycine and proline	Gram-negative bacteria	No	Lee et al. (1994)
Apis mellifera	Hymenopteacin	93		bacteria	No	Casteels et al. (1993)
Hyalophora.cecropia. Helicoverpa armigera. Trichoplusiani	Gloverin		α helical	Gram-negative bacteria	No	Axén et al. (1997), Mackintosh et al. (1998a) and Lundström et al. (2002)
Acolaepta luxuriosa.	Acaloleptin	71		Gram negative bacteri	No	Imamura et al. (1999)
			Vertebrates			
Bos Taurus	Indolicidin	13		Bacteria, Viruses, Fungi	No	Selsted et al. (1992)
Synthetic Indolicidin	Omiganan			Bacteria, Fungi	Failed phase II trials	Isaacson (2003)
Homo sapiens	Lactoferricin-B		Tryptophan rich	Bacteria, Fungi	No	Bellamy et al. (1992)

This resulted in peptides that were no longer hemolytic, but were very effective against tumor cells (Papo and Shai, 2003). Another mellitin analogue, hecate, was found to be toxic to breast cancer cells (Leuschner et al., 2003). Hecate's effectiveness has been increased by creating hecate hormone conjugates. By conjugating hecate to hormones, whose receptors are found on the surface of cancer cells e.g. luteinizing hormone, the cell selectivity of hecate can be increased (Hansel et al., 2007).

The naturally occurring alloferon, as well as synthetic alloferon from the blow fly *C. vaicin,* was able to slow tumor growth. It was, however, unable to eliminate cancer cells at high concentrations (Chernysh et al., 2002).

Application to transgenics

The tobacco budworm *H. virescens,* and the fall webworm *H. cunea* are both agricultural pests (Ourth et al., 1994; Park et al., 1997). Research into the responses of these insects to microbial infections could be important in the future control of their populations. Methods could be designed to inhibit insects defense, and increase the rates of mortality in the field (Ourth et al., 1994; Park et al., 1997).

Conversely, the cultivation and conservation of useful insect species could be boosted by increasing the defenses these insects have against pathogenic microorganisms (Destoumieux et al., 1997). Research into the structure and genetics of immune peptides may ensure the long-term survival and continued use of these insects (Destoumieux et al., 1997).

Concerns have been expressed about the levels of antimicrobial peptides present in domesticated poultry, due to intense breeding and in some cases in-breeding (Joerger, 2003). A decrease in the levels of antimicrobial peptides may leave breeding and domestic populations at the risk of microbial infections. Fisheries and fish breeding projects are also significantly adversely affected by infectious diseases caused by pathogens such as *Vibrio anguillarum.* Consequently, pilot studies have been performed on the use of cecropin-mellitin hybrids (CEME) to combat infections in Coho Salmon (Jia et al., 2000). This synthetic peptide caused no adverse effects when it was injected into young salmon. When fish were infected with lethal doses of *V. anguilarum* the mortality rate in the fish that received CEME treatment was 4 to 5 times lower than that in the control group (Jia et al., 2000).

This approach could also be applied in useful crops via insertion of genes encoding antimicrobial peptides into their genomes, thus providing the plants with defense mechanisms against various fungal and bacterial pathogens. Indeed transgenic tobacco expressing heliomicin and drosomycin was found to have higher resistance to fungal infections (Banzet et al., 2002).

Antimicrobial peptides as drug delivery systems

Drug delivery molecules need to be able to penetrate thetarget cells, while displaying low levels of toxicity. The hemipteran peptide pyrrhocoricin has been investigated as a potential drug delivery tool due to its low toxicity to eukaryotic cells, as well as the ease with which it penetrates bacterial as well as human cells. It was shown that the peptide and a synthetic pyrrhocoricin analogue could penetrate human fibroblasts and dendritic cells (Otvos et al., 2004). This feature of the peptide to enter but not kill eukaryotic cells is due to its mode of action, which is described below.

The mode of entry for the short glycine rich peptides apidaecin and drosocin is expected to be the same for pyrrhocoricin (Kragol et al., 2001). All three of these peptides have been found to enter the target cell and become rapidly distributed in all cellular compartments (Kragol et al., 2001). The initial step of the entry of these peptides into bacterial cells is expected to involve their association with some component of the outer cell membrane. After successful invasion of the periplasmic space the peptides then bind to some inner membrane component (Castle et al., 1999). However, this association does not destabilize the plasma membrane or result in the permeabilisation of the target cells (Otvos et al., 2004). The peptide is then translocated into the cell where it interacts with specific targets. Pyrrhocoricin interacts with the bacterial chaperonins and heat shock proteins GroEl and DnaK. The pyrrhocoricin binding site on DnaK has been identified to be the hinge region of the protein's multihelical lid that covers the peptide-binding site (Kragol et al., 2001). Upon binding pyrrhocoricin prevents the helical lid from opening and this leads to inhibition of protein folding. Pyrrhocoricin also inhibits DnaK's ATPase activity, which further narrows the probable binding site to one of the helices between helix D and E (Kragol et al., 2001).

Control of insect borne diseases

There are many insects that act as vectors of parasites that have a major effect on human health, crop plants and domestic animals. The historical method of disease control has been through the control of the insect vector (Durvasula et al., 1997). These methods have become less effective due to an increasing emergence of insecticide and drug resistant insects. Even biological methods of control, such as sterile male release are less effective than they once were (Mogi et al as cited by (Durvasula et al., 1997). One of the strategies to control these diseases is to develop methods that reduce ability of the parasite or pathogen to infect the insect host. This can be achieved by genetically modifying the immune response of the vector, or through the use of transgenic symbiotic bacteria (Boulanger et al., 2001) (Durvasula et al., 1997).

The mosquito, *A. gambiae* is the principal vector of human malaria in Africa. The number of cases of mosquito-transmitted malaria is increasing resulting in about 3 million deaths per year. Mosquitoes also spread lymphatic filariasis and arboviruses such as Dengue fever and yellow fever (Lowenberger, 2001). Despite the high transmission rate in the field, the vast majority of parasite and mosquito contact do not result in infection. This is also true in the tsetse fly *G. moristans* where infection rates are low (Boulanger et al., 2001). *Plasmodium bergei* elicits an immune response in the form of antimicrobial peptides in *A. gambiae,* and *A aegypti*, while *Trypanasoma brucei brucei* elicits a similar response in *G. moristans moristans* (Boulanger et al., 2001). The development of *P. bergei* in Anopheline mosquitoes can be stopped by administering exogenous cecropin to the mosquito (Gwadz et al., 1989). Another strategy being investigated is the use of the scorpion venom peptide scorpine. Scorpine was found to block the development of malaria parasites in tissue culture , as well as in the gut of transgenic fruit flies (Possani et al., 2002).

Further evidence for this line of thought is demonstrated by the peptide stomoxyn. Despite living in the same area, being exposed to the same pathogens and having similar physiology, *S. calcitrans* is not a vector for trypanosome parasites, while the tsetse fly *G. moristans* is (Boulanger et al., 2002). The immune response of these insects was compared. Both insects release defensins into the anterior region of their gut. However, stomoxyn is unique to *S. calcitrans,* and could not be identified in the tsetse fly (Boulanger et al., 2002). By creating a strain of tsetse fly that can express this peptide within its gut, the number of effective vectors for the trypanosome parasite could be decreased.

It is due to these facts that the strategies of transgenic insect vectors expressing enhanced and more effective forms of cecropins (Boulanger et al., 2001), or the use of genetically transformed bacteria expressing antimicrobial peptides (Durvasula et al., 1997) are being investigated. The generally accepted strategy to accomplish this would be to place the transcription of the antimicrobial peptide under the control of the promoters for the transcription of proteolytic gut enzymes in the transgenic mosquitoes (Possani et al., 2002). However, ecologists have pointed out problems with using transgenic mosquitoes. These include doubts as to whether the transgenic mosquitoes could effectively compete with wild type mosquitoes for mates or resources (Enserink, 2002). However, male crickets are able to advertise their pathogen resistance ability to females using their calling song. Males with a higher chirp rate in their song were found to possess increased pathogen resistance. Therefore any males with a more effective immune response should be able to out-compete any other males (Ryder and Siva-Jothy, 2000). Unfortunately, this may become a problem in an area with limited resources, as it is thought that the mounting of an immune response by the host insect is costly and must be compensated for by an increased resource intake (Moret and Schmid-Hempel, 2000).

A solution to this problem could be to imitate the *D. melanogaster* attacin, where the processing of the immature peptide gave rise to the mature attacin, and a short proline rich peptide (Asling et al., 1995). This means that the insect is getting two peptides for the price of one, and both peptides are still being properly secreted. Another problem ecologists have pointed out is the ability of *P. falciparum* to develop resistance to the host resistance transgenes (Enserink, 2002). However, if the resistance gene is an enhanced antimicrobial peptide that is still under the control of the recognition pathways of the innate immune system, it may not be that easy for *P. falciparum* to develop resistance to these genes. Finally, there is the problem of multiple vectors in a given area. In these areas transgenic members of each of these species would have to be released into the area (Enserink, 2002). Bacterial symbionts of insect vectors of diseases have been engineered to express antimicrobial peptides. The assassin bug *R. prolixus* is the main vector of Chagas disease, which is spread by the parasite *Trypanosoma cruzi.* Transgenic bacterial symbionts of *R. prolixus* have been transformed to express cecropin (Durvasula et al., 1997). The expression of this peptide resulted in the total elimination of the parasite, or at least in reduction of its number. Any negative effects such as toxicity to gut flora or insect cells do not appear to be a problem (Durvasula et al., 1997).

OBSTACLES TO THERAPEUTIC USE

Obstacles to the commercial use of antimicrobial peptides include high cost, difficulties in mass production, loss of activity under physiological conditions, potential toxicity, peptide aggregation, peptide stability (Marr et al., 2006) and newer, effective conventional antibiotics (Gordon et al., 2005). These conventional antibiotics represent a safer more trustworthy drug development option for pharmaceutical companies. In addition to these problems very few antimicrobial peptides have received FDA approval. This has resulted in many pharmaceutical companies abandoning antimicrobial peptides (Gordon et al., 2005).

Cost

One of the many strategies used to lower the cost of production is to use smaller peptides (Marr et al., 2006). This has led to the design of synthetic and model antimicrobial peptides. Model amphipathic peptides have been constructed to optimise the effect of the a-helices involved in the disruption and channel formation within bacterial cell membranes (Blondelle and Houghten, 1992). Cecropin-mellitin hybrids have been designed in order to maximise the active range of peptides, while minimising its effect on the host's cells. The hybrids are small molecules that minimize immunogenicity and

Table 4. Unusual insect antimicrobial peptides that do not fall into any category. Three unusual peptides have been isolated from insects, which cannot be grouped into any class or group. The jelleines appear to be secondary metabolites from mastoporan processing. They and the alloferons are two of the smallest antimicrobial peptides that have been isolated.

Sources	Name	Size amino acid	Activity	Commercialised or not	References
Calliphora viciana	Alloferon	12-13	Cancer Virus	No	Chernysh et al. (2002)
H. virescens	Viresin		Gram negative bacteria	No	Chung and Ourth (2000)
Apis mellifera	Jelleines	8-9	Bacteria and yeast	No	Fontana et al. (2004)

expense of synthesis (Boman et al., 1989). Additionally synthetic oligopeptides based on the structure of the *A. dichotoma* defensin have been synthesised and some of these analogues have greater activity against *S. aureus* than the original peptide (Yamada et al., 2005). These hybrid peptides were found to be more effective antibacterial and antimalarial peptides, and they did not lyse mammalian red blood cells (Boman et al., 1989).

In order to lower the costs of production, smaller synthetic peptides are being created. One strategy being used is to extract active linear fragments from insect defensins (Lee, 2002). Tenecin 1 is a defensin isolated from *Tenebrio molitor*. An active fragment corresponding to the β sheet region was successfully purified. In addition to this the α helical region of tenecin 1, which was originally inactive, could be transformed into an active peptide by single and double amino acid substitutions to increase the positive charge of the peptide (Ahn et al., 2006). In addition to these shortened peptides being easier to produce, they may also present lower levels of antigenicity (Lee, 2002), and higher levels of activity (Boman et al., 1989) than the natural parent peptide.

Insects possess many antimicrobial peptides that are naturally small and should be cost effective to synthesize. These include the alloferons as well as the jelleines (Table 4).

Production

Difficulties around mass production of antimicrobial peptides are a major obstacle in the use of these peptides as therapeutic agents. Biological expression of antimicrobial peptides in bacteria is difficult and normally, failure can be attributed to the recombinant protein being inactive or the protein being toxic to the bacterial cell. Attempts to produce pilosulin in *Escherichia coli* resulted in an inactive protein being produced (Inagaki et al., 2004). However, active human β defensin has been successfully produced in *E. coli* as a GST-Fusion protein (Si et al., 2007). This is surprising as the defensins require the formation of disulfide bridges. The insect peptide moricin from the silkworm *Bombyx mori* was a perfect candidate for production in bacteria. Firstly, it contains unique structural elements at its C-terminus, making chemical synthesis difficult (Hara and Yamakawa, 1995). Secondly, it contains no disulfide bonds and should therefore fold properly in bacteria. This led to moricin being successfully produced using two separate protein fusion systems (Hara and Yamakawa, 1996).

A possible solution to the difficulties experienced in the mass production of antimicrobial peptides may be the fungal defensin plectasin. Plectasin was isolated from the saprophytic fungus *Psuedoplectania nigrella*. Plectasin resembles arachnid and primitive insect defensins in structure; it shows high activity against bacteria and low levels of cytotoxicity. Being produced in a fungus meansthat plectasin could be produced in high amounts (Mygind et al., 2005a).

Proteolysis, inactivation and toxicity

Due to the high content of basic residues found in most antimicrobial peptides they would be prone to degradation by trypsin-like proteases. Insect pathogenic strains of *P. aeruginosa* are able to avoid antimicrobial activity by degrading cecropin using a cecropin specific protease activity (Jarosz, 1997). Additionally, cecropin degrading enzymes from *Bacillus larvae* were able to effectively remove cecropin activity from the hemolymph of honeybees (Jarosz and Gliński, 1990).

Interest has been shown in the use ofpyrrhocoricin to treat infections because of its low toxic effects on mammalian cells. Furthermore, pyrrhocoricin is more resistant to degradation in mammalian sera than many other peptides (Otvos et al., 2000). Studies on the *in vivo* effectiveness of pyrrhocoricin demonstrated that it was able to protect mice from an *E.coli* infection. At high doses, however, the peptide killed the mice (Otvos et al., 2000). Derivatives were designed to decrease toxicity and increase antimicrobial activity. The most effective of these pyrrhocoricin derivatives contained unnatural amino acids at both termini, and was more resistant to the proteolytic actions of the mouse sera (Otvos et al.,2000).

Another strategy to improve the resistance of antimicrobial peptides to the action of proteases is the use of D enantiomers (Papo and Shai, 2003). This strategy can only be used with membrane lytic peptides that do not interact with specific targets. Good initial results have already been demonstrated using this strategy with mellitin D, L derivatives (Papo and Shai, 2003).

Serum, culture media as well as the physiological

Table 5. Antimicrobial peptides derived from bacteria. Summary of some of the antimicrobial peptides isolated from bacteria and fungi. The table gives details of the peptides structure, range of activity and the current status of any attempts to commercialise the peptide or its derivatives.

Sources	Name	Structure	Activity	Commercialised or not	Reference
Actinoplanes sp.	Ramoplanin	Cyclic lipopeptide	Gram-positive	No	Cavalleri et al. (1984)
Streptomyces roseosporus	Daptomycin	Cyclic lipopeptide	Gram-positive	Yes	Eliopoulos et al. (1986)
Bacillius polymyxa	Polymixin B		Gram-positive	Yes	Watt and Vanderift (1950)
Lactococcus lactis	Nisin	Cyclic lipopeptide	Gram-positive	Yes	Berridge (1949)
Leuconostoc gelidum	Leucocin	Cyclic lipopeptide	Gram-positive		Hastings et al. (1991)

concentrations of salts have been reported to inhibit the activity of antimicrobial peptides (Johansson et al., 1998). This is probably due to the presence of NaCl and divalent cations. Once again this problem may be overcome through peptide engineering. A synthetic version of the human antimicrobial cathelicidin peptide LL37 named WLBU2 was able to kill *S. aureus* in an isotonic saline environment and continued to kill the bacteria in environments with increasing salt concentrations. LL37 was inactive at all tested salt concentrations (Deslouches et al., 2005).

RESISTANCE TO ANTIMICROBIAL PEPTIDES

However, there are bacteria defense mechanisms against antimicrobial peptides. For instance attacins and cecropin are degraded by a protease from *Bacillus thuringeiensis* (Dalhammar and Steiner, 1984). This was further demonstrated by the finding that when this protease is injected into the hemolymph of the tsetse fly *G. moristans.* The flies become more sensitive to bacterial infections (Kaaya et al., 1987).

The dangerous pathogenic *S. aureus* is able to resist high concentrations of many membrane permeabilising peptides by incorporating a higher degree of positive charges into its bacterial membrane. Since these peptides operate by using their positive charge to associate with the negatively charged membrane, an increase in the positive charge of the membrane makes it difficult for the peptides to associate with the membrane (Peschel et al., 1999). The bacteria are able to do this via the *dlt* gene products, which allows the incorporation of D-alanine into the cell wall teichoic acids (Peschel et al., 1999). This means that *S. aureus* strains carrying a mutation in the *dlt* operon are more sensitive to the positively charged antimicrobial peptides.

Another strategy adopted by bacteria to change their outer membrane, is the incorporation of a large concentration of lysine residues in the outer membrane. This would also change the charge of the membrane to a more positive charge (Wang et al., 2002). Fears have been raised that the use of antimicrobial peptides based on human natural defense peptides may result in increased resistance of pathogens to our own immune system (Bell and Gouyon, 2003). There has been no indication that this has been the case with peptides that have been on the market for many years such as nisin (Hancock, 2003).

PEPTIDES UNDER CLINICAL DEVELOPMENT

Many peptides are already present in over the counter medicines and in foods. Polymoxin B is present in topical creams and eye ointment while Nisin has been used as a preservative in food since the late 1960s (Table 5). Additional peptides in clinical development are listed in Table 6.

CONCLUSION

Despite the problems associated with the production and cost of antimicrobial peptides, we now know enough concerning structure and design to create novel synthetic peptides that show little or no cytotoxicity to non-cancerous human cells. These synthetic peptides may also overcome the inactivation of antimicrobial peptides *in vivo.* Shortened synthetic peptides have the added advantage of being cheap to produce.

It is also interesting to note that only Iseganan failed clinical trials due to lack of activity. Pexiganan was refused approval by drug agencies, not due to lack of performance, but because it was no more effective than present treatments. The decision to refuse approval of pexiganan reflects continued faith in older traditional antibiotics, more than it does the failure of the drug as an effective antimicrobial.

Insects represent a rich resource of antimicrobial peptides, many of which are extremely small and therefore cheap to synthesise. Others, such as moricin, can be mass produced in bacteria. The success of the synthetic mellitin analogue hecate and the cecropin derivative D2A21 in the treatment of bacterial infections, breast cancer, and viral inhibition, shows that these peptides are a promising avenue for drug development.

Table 6. Antimicrobial peptides undergoing clinical development.

Peptide	Origin	Stage of development	Activity (target)	Results of trials	References
D2A21	Cecropin analogue	Phase I trial testing	Treatment of infected wounds.	Found to be efficient in preventing burn infections	Schroder and Harder (2006)
Insect defensins (ETD151)	Heliomycin derivative	Pre-clinical trial testing	Treatment of fungal infections.	Promising results	Andres and Dimarq (2005)
Insect defensin	E.g dragonfly defensins	Pre-clinical trial testing	Gram positive bacterial infections.		Andres and Dimarq (2005), Bulet et al. (1992)
Pexiganan	Synthetic derivative of magainin	Phase III trial testing	Pathogenic infections of diabetic foot ulcers	Drug was refused FDA approval, on the basis that is was no more effective than conventional antibiotics	Moore (2003)
Iseganan	Protegrins.	Abandoned	Bacterial infections	Failed to show any promising result	Gordon et al. (2005)
Omiganan	Synthetic analogue of indolicidin	Phase III trial testing	Active against bacteria and fungi	Failed to prevent infections, but it was able to decrease the instances of catheter tube colonization by bacteria.	Gordon et al. (2005)
MBI 594AN	Cathelicidin, based peptide	Treatment of acne	Phase II trials	Highly effective in reducing inflammation and the formation of lesions	Gordon et al. (2005)

Additionally the ability to isolate smaller active components of insect antimicrobial peptides would lead to lower production costs.

Finally, developing drugs based upon arthropod antimicrobial peptides would partially remove the concern for pathogens developing increased resistance to these peptides. If we base antimicrobial peptide drugs upon mammalian peptides we run the risk of giving pathogens an increased opportunity to develop resistance against our own innate defense peptides (Bell and Gouyon, 2003).

As antibiotic resistance becomes an increasing threat the value and potential of antimicrobial peptides as a new source of antimicrobial compounds increases. This combined with other potential uses such as their ability to act synergistically with conventional antibiotics, their ability to prevent endotoxic shock and the difficulty pathogens have in developing resistance, makes antimicrobial peptides an important source of future chemotherapeutic agents.

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