

**INTRODUCTION OF NEW ANTIMICROBIALS: CATIONIC PEPTIDES**

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ABSTRACT

Antimicrobial cationic peptides are of interest because they can combat multi-drug-resistant microbes. Most peptides form α -helices or β -sheet-like structures that can insert into and subsequently disintegrate negatively charged bacterial cell surfaces. Here, we show that a novel class of core-shell nanoparticles formed by self-assembly of an amphiphilic peptide has strong antimicrobial properties against a range of bacteria, yeasts and fungi. The nanoparticles show a high therapeutic index against *Staphylococcus aureus* infection in mice and are more potent than their unassembled peptide counterparts. Using *Staphylococcus aureus*-infected meningitis rabbits, we show that the nanoparticles can cross the blood-brain barrier and suppress bacterial growth in infected brains. Taken together, these nanoparticles are promising antimicrobial agents that can be used to treat brain infections and other infectious diseases.

KEYWORDS: antibiotic, bacteria, Membrane, helices, Cationic Peptides

INTRODUCTION:

The discovery of penicillin in 1929 by Alexander Flemming was a milestone in modern medicine.¹ In 1943 the industrial-scale fermentation (production) of penicillin enabled its widespread use and ushered in the beginning of the "antibiotic era." The availability of antibiotics has had a profound effect on human health and has contributed to an eight-year increase in the average human lifespan.² The availability of antibiotics has allowed the successful treatment of many bacterial infections as well as the ability to perform invasive medical procedures including surgery and chemotherapy.³ Throughout the years, however, many bacterial strains have evolved ways to adapt or become resistant to the currently available antibiotics.³

An example is *Staphylococcus aureus*. More than 95% of the strains of these bacteria are resistant to the antibiotic penicillin.⁴ This is problematic since very few new families of antibiotics have been discovered in the past 30 years.³

One of the reasons that antibiotic resistance is so widespread is the pervasive use of antibiotics in animal feeds to prevent infections and promote growth.⁵ This has encouraged spontaneous mutation of the targets of antibacterial drugs as well as the exchange of plasmids encoding antibiotic resistant genes.⁴ There are many mechanisms by which bacteria evade antibiotic treatment:

- reduced drug uptake
- active pumping of drugs out of the cell
- enzymatically altering the antibiotic
- modification of targets
- drug sequestering by protein binding
- overproduction of the target
- metabolic bypass of the targeted pathway⁴

Antibiotics comprise an estimated 7 to 22 billion dollar a

year industry and are the third largest class of drug sold.⁴ There are currently over 150 antimicrobial drugs that have been approved for use in the United States as well as approximately 27 in clinical development.⁴ It is likely that many of the new antibiotics in development will not be approved by the US Food and Drug Administration as only a handful have been approved in the last 10 years.⁴ Most of the drugs in development are analogs of earlier antibiotics which work on a select number of bacterial targets.⁴ There is an increasing demand for novel drugs to combat bacterial infections.⁶

One approach to this problem of resistance to current antibiotics is to look to the "evolutionary ancient weapons."⁷ All multicellular organisms have evolved in the presence of a plethora of microbes throughout the years, some without the protection of antibodies or immune effectors cells.⁷ On a daily basis, we are exposed to a number of pathogens through ingestion or contact with infected surfaces.⁸ The reason we continue to survive is due to the effectors of our innate immune response producing a non-specific broad spectrum response to injury and infection.⁹ All organisms possess a variety of broad-spectrum antimicrobial peptides known as cationic peptides.⁷ Microbial pathogens don't seem to acquire resistance to these cationic peptides even though they have been exposed to them for a very long time.⁷ For this reason there has been a lot of commercial interest and effort in developing cationic peptides as potential antimicrobial therapeutics.⁷

CATIONIC PEPTIDES:

Approximately 20 years ago, it was discovered that the skin of frogs, the lymph of insects, and human

neutrophils (a type of white blood cell) all contain cationic peptides (Figure 1) that can act as potent antimicrobials.³ To date, more than 600 cationic peptides have been discovered in virtually all organisms from microbe to man. These cationic peptides have a broad spectrum of antimicrobial activity including activity against bacteria, eukaryotic parasites, viruses, and fungi.³ the same cationic peptide sequences are rarely recovered from different species, even those that are closely related.⁷

Cationic peptides vary considerably in sequence and structure, with a few common features.^{10,11} All cationic peptides are encoded as larger precursors with signal sequences that are subsequently modified by cleavage or addition of groups like glycosylation or halogenation.⁷ They range in size from 12-50 amino acids and have molecular masses of less than 10000.^{3,6} Cationic peptides are amphipathic meaning they possess both a hydrophobic region that interacts with lipids and a positively charged hydrophilic region that interacts with water or negatively

charged residues.^{3,6} This feature allows the peptides to interact well with membranes that are composed of amphipathic molecules, especially negatively charged bacterial membranes.³ For the most part, animal cells tend to have membranes with no net charge so they are unaffected by cationic peptides.⁶ There are four major classes of cationic peptides³:

- β -sheet molecules stabilized by two or three disulphide bonds
- amphipathic α -helices
- extended molecules
- loops with a single disulphide bond

Cationic peptides have been found to have activity Against Gram negative and Gram positive bacteria as well as fungi, eukaryotic parasites, and viruses.³ most importantly, cationic peptides seem to be effective against many strains of antibiotic resistant bacteria.³

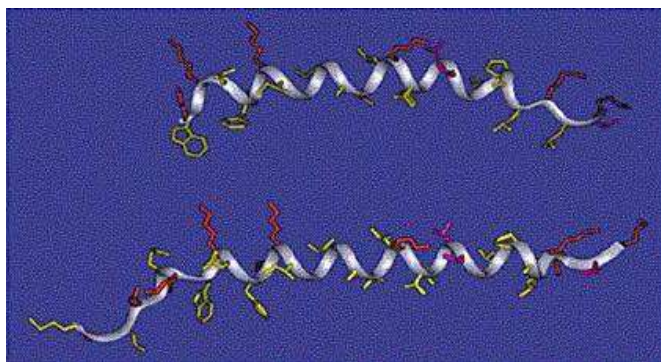


Figure 1:

MECHANISMS OF ACTION:

Cationic peptides seem to interact with bacterial membranes.³ Bacterial cells may have two membranes on which cationic peptides can act:

- the outer membrane
- the cytoplasmic membrane

1. ACTION OF CATIONIC PEPTIDES ON OUTER MEMBRANE:

In Gram negative bacteria, it is hypothesized that the cationic peptides interact with the highly negatively charged outer membrane. The surface of the membrane contains magnesium ions which function to neutralize the charge. It is believed that the cationic peptides displace these magnesium ions³. The peptides then either bind tightly to the negatively charged membrane lipopolysaccharide (LPS) or neutralize the charge over an area of membrane, subsequently distorting the membrane structure.³ Once this is accomplished, the peptides can translocate across (that is, insert themselves into) the outer membrane.

2. ACTION OF CATIONIC PEPTIDES ON CYTOPLASMIC MEMBRANE:

The bacterial cytoplasmic membrane is also negatively charged. Cationic peptides can insert in a position parallel to the membrane lipids and fold into membrane-bound structures leading to four possible outcomes:³

- cell lysis, although this is not very likely at physiological concentrations of cationic peptide
- channel formation, where the peptides reorientate themselves in the cytoplasmic membrane in such a way that they form channels leading to a breakdown in membrane integrity
- the carpet model where cationic peptides cause the catastrophic breakdown of the membrane integrity
- direct killing of the bacterial cell by peptides attacking internal targets like cellular polyanions (for example, nucleic acids) in the cytoplasm after diffusing through both membranes

ROLES IN IMMUNITY:

Cationic peptides not only directly kill bacteria, they also have profound effects on the hosts system.^{8,12-14} It has been shown that cationic peptides can function to sequester LPS shed from Gram negative bacteria. This LPS functions as an endotoxin and may lead to sepsis.³ Cationic peptides have also been found to be potent chemo-attractants, recruiting antigen-presenting cells, which can initiate responses by the adaptive immune system.¹⁵ They have also been known to recruit mast cells (a type of white blood cell) which are known to increase blood vessel permeability. This increased permeability allows other immune effectors and wound healing factors to permeate injured tissues.¹⁵

IN THE CLINIC:

In order to be a good candidate for therapeutic use, a drug needs to show good activity, appropriate function, and low toxicity, have stability in vivo and be reasonably inexpensive to manufacture.³

So far only a few cationic peptides have made their way to clinical trials and only two are used in topical creams and solutions.³ one of the problems that limit the systemic use of these compounds is toxicity.³ currently research is being done to structurally alter these compounds to make them less toxic to the consumer.

Another problem associated with administering cationic peptides as a treatment for infection is the ability to direct the peptides to the appropriate locations with the accuracy of white blood cells and epithelium.⁶ When injected intravenously the peptides are required to infiltrate healthy tissue in order to reach the appropriate locations, which can be a very slow process.⁶ The host's system can also act on the peptides; for example, the presence of proteases can inactivate peptides before they reach their destination.³ Finally, sometimes the activities of the peptides are not the same in vitro and in vivo. For example one of the most potent cationic peptides studied to date in vitro, polyphemusin I from horseshoe crabs, has been shown to have no activity against infections in animal models.¹⁶

Finally, cationic peptides are also very expensive to produce because they can't be mass-produced in bacteria or fungi the way many other antimicrobial drugs are produced. Consequently, the cost of treatment is estimated at approximately \$100 a day.³

APPLICATIONS IN BIOTECHNOLOGY:

Cationic peptides have a number of applications in biotechnology. One application for cationic peptides is the formation of transgenic plants. Phytopathogens are a major problem in cultivated and stored crops leading to the

loss of between 30-50 billion dollars annually.¹⁷ Crop spoilage also increases the incidence of carcinogens and has an adverse effect on the health of the consumer.¹⁸ Pesticides and antimicrobials are not really a good way to combat phytopathogens because they can be expensive, detrimental to the environment and harmful to the consumer.¹⁸ They also can lead to the development of resistant bacterial strains. It is hypothesized that by producing transgenic plants that express cationic peptides with broad specificity, many of these problems will be circum-vented.¹⁸ Preliminary results from the University of Victoria indicate that this approach may be useful.¹⁸

Microgene International Ltd. is currently using the technology to engineer genetically enhanced fish that over-produce cationic peptides. The hope is that these fish will not be as susceptible to infections. Normally 25% of aquaculture stock is susceptible to infections and consequently lost.¹⁹

Another potential application is the formation of aerosol sprays for patients with cystic fibrosis.¹⁹ Cystic fibrosis is a disease caused by a genetic mutation in the CFTR gene. This mutation leads to the inability to regulate chloride ion concentrations across membranes. The buildup of high chloride salt concentration in the lungs reduces the ability of the CF patients' cationic peptides to function.¹⁹ These individuals then become more susceptible to infections by opportunistic bacteria like *Pseudomonas aeruginosa*.¹⁹ Through molecular biological techniques it may be possible to engineer cationic peptides in such a way that they will be less susceptible to high salt concentrations.¹⁹

CONCLUSION:

As bacteria are becoming resistant to conventional antibiotics researchers are looking for new ways to combat microbial infections.²² One possibility is the development of cationic peptides as commercial antimicrobial agents. Cationic peptides are present in all organisms and function in the killing of bacteria, viruses, eukaryotic parasites, and fungi. Bacteria don't seem to acquire resistance to the cationic peptides, which make them an attractive subject for drug re-search. Another plus is that they vary between organ-isms, which could lead to a greater selection of drugs. It may also be possible to transgenically alter plants and animals in agriculture so that they have increased levels of cationic peptides in an attempt to reduce losses. There are still many barriers in the develop-ment of cationic peptides as commercial antimicrobial agents. However, with time and future research, perhaps they may provide a feasible option for conquering microbial infections.²⁴

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