NOVEL ANTIMICROBIAL PEPTIDES FOR THE TREATMENT OF ORTHOPAEDIC INFECTIONS

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Introduction

Even with recent advances in diagnosis, antibiotic developments and refined surgical techniques, the management of orthopaedic infections remains a challenging clinical problem. Antimicrobial resistance often results in the need to use new antibiotics. Bacteria become increasingly insensitive to antibiotics, consequently early and aggressive surgical treatment gains importance. The current treatment options are surgical debridement, 4 – 6 weeks systemic antibiotic treatment, lavage, and local antibiotic treatment. In the last decade, a number of antimicrobial compounds have been defined, the antimicrobial peptides. Since then many antimicrobial peptides have been investigated such as magainins on frog epidermis, cecropins in pigs, and defensins in mammals.

Antimicrobial peptides possess various structural characteristics, including amphipatic alpha helices and beta sheets. They are usually catonic and highly hydrophobic, and often described as surface seeking substances. Molecular modeling led us to a new substance, which is similar in secondary structure to the antimicrobial peptides mentioned above but has no obvious amino acid homology. This peptide derives from the envelope of the HIV–1 virus. Previous studies have shown that our peptide family has little cytotoxicity, and is highly selective, broad-spectrum antimicrobial agents which can be potentially used as new weapons for the cure of diseases caused by a wide variety of microbial pathogens. With this study, we investigated the feasibility of the peptide in the field of joint infections.

Methods

Peptide Synthesis. Peptides were synthesized as C-Terminal amides with Advanced Chentech 200 (Advanced Chentech, Louisville, Ky.) automated peptide synthesizer. In a second step, the peptides were purified and quantified.

Bacterial killing tests and test for erythrocyte lysis. Bacterial killing tests included staphylococcus aur. The bacterial killing assay and the cytotoxicity testing were conducted as described previously.1

In vivo experiments. Three month old, 2.5 lb. New Zealand white rabbits were anaglo-sedated (30mg/kg Ketamine and 2.5 mg/kg acepromazine) for injection of the knee joints through the patellar tendon with a 22 gauge needle. The policies and procedures of the animal laboratory are in accordance with those detailed by the USA Department of Health and Human Services. The research protocols used for these experiments were approved by the Animal Research and Care Committee (ARCC) at the authors’ institution.

In the first set of experiments, we inoculated each knee with a solution of 8 µM antimicrobial peptide. After 1 hour, colony counting was achieved. At two hours, colony counting increased again (Fig 2).

Discussion

The major goals for antimicrobial peptides are antibacterial potency, selectivity or low cytotoxicity, and activity under physiological conditions. In vitro we proved the high antibacterial potency of our antimicrobial peptide in physiologic saline concentrations and in the presence of synovial fluid. The potency in presence of physiologic saline concentrations represents a major advantage over other antimicrobial peptides.

The cytotoxicity is minor in comparison to its potency. It remains to be investigated whether the decrease in potency in presence of synovial fluid also decreases the cytotoxicity. However, in the knee joint, no inflammation could be seen macroscopically.

We regard the knee joint model for infection as useful for the measurement of the parameters mentioned above. Repeated knee joint aspirations can be performed reliably, as well as help to minimize bias from outside. Although joint infections are not the only imaginable clinical use, they are a high consequence event in orthopedics.

The in vivo data suggests that the biologic half life of our antimicrobial peptide is short. A continuous instillation or liberation of our substance is a prerequiste for its efficiency. Since 1) local antibiotics is a treatment option in septic surgery and 2) new strains of antibiotic drug resistant bacteria regularly appear, future developments in the field of antimicrobial peptides are worth pursuing. Gene therapy, removable implants or bioresorbable implants with a long term expression of this antimicrobial peptide might open a new era in the treatment of chronic, surgically incurable infections.

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Reference List

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