INTRODUCTION Current treatment of osteomyelitis usually includes a thorough debridement and placement of polymethyl methacrylate beads releasing an antibiotic e.g. gentamycin. As the induction of gentamycin resistant bacteria in patients treated with gentamycin has recently been reported(1), new treatment options, including novel antibiotics which are slowly released from resorbable carriers, need to be explored.

Antimicrobial peptides (AMP) are an essential part of the innate immune system, they have both a rapid antimicrobial killing mechanism and a low or nonexistent capacity to induce resistance. These peptides have been proposed as a novel class of natural antibiotic agents that are active against drug resistant bacteria. AMP might be used clinically to treat infections caused by resistant bacterial strains(2). The antimicrobial peptide hLF1-11 consists of amino acids 1-11 of human lactoferrin, and has a broad antimicrobial spectrum: it has shown in vivo bactericidal activity against a variety of micro-organisms, including multi-drug resistant Staph. aureus(3). Bioresorbable calcium phosphate (CaP) cements can be used as a carrier for the controlled release of antibiotics(4). We studied the efficacy of CaP-loaded CaP cement and gentamycin-loaded CaP cement for the prevention of osteomyelitis in a rabbit model.

METHODS In total, 24 rabbits were operated aseptically on the right femur, as described by Nijhof and coworkers(5). The rabbits were divided into four groups: Control, AMP, Gentamycin (all n=7) and Sham (n=3). After surgical exposure, a hole was drilled through the cortex at the trochanter tertius. The femoral canal of the rabbits in the Control, AMP and Gentamycin groups was inoculated with 10^6 CFU Staph. aureus Wood 46 (non-resistant strain), the Sham group was not infected. Immediately afterwards 0.5 ml CaP cement (Bonesource, Stryker-Leibinger, Freiburg, Germany), was injected into the femoral canal. The cement for the AMP group contained 50 mg hLF1-11 per gram cement powder (AM-Pharma, B.V., Bithoven, the Netherlands), for the Gentamycin group 50 mg Gentamycin-Sulphate per gram cement powder (Bionet Merck, Darmstadt, Germany). No antibiotic was added to the cement in the Control and the Sham group.

Postoperatively, A-P and Lateral X-Rays were obtained. At 21 days following the surgery; the animals were euthanised, X-rayed and the femora were excised aseptically. A cylinder of bone was sawn off and cleaned of cement, ground in phosphate buffered saline, serially diluted and cultured; colony forming units (CFU) were counted. The lower detection limit was 2.5E+3 CFU/gr bone. A two tailed t-test was used to compare results between groups, p<0.05 was considered significant.

The X-Rays were scored for signs of osteomyelitis by two independent observers, using the system described by Norden(6), sequestra, bone formation and destruction were scored (0; no change, 7; maximal osteomyelitis score), the percentage of cement filling the femoral cavity was also estimated.

ESSENTIAL RESULTS One rabbit of the Gentamycin group died immediately after surgery; necropsy was performed, probable cause of death: fat embolisms in the lungs. The other rabbits recovered well.

Average culture results per infected group are shown in Figure 1. All cultures of the Sham group were sterile. Bacteria were cultured from the infected femur in six of seven Control rabbits, in four of seven AMP rabbits, and in one of six Gentamycin rabbits, all other cultures were negative. Two of the four positive cultures in AMP rabbits were at the lower detection limit. A significant difference in CFU was observed between AMP and Control (p=0.047) and between Gentamycin and Control (p=0.043), no significant difference was found between AMP and Gentamycin.

X-Ray changes in a femur with positive bacteria cultures (Control-group) are shown in Figure 2A, a femur without changes (AMP-group) is shown in Figure 2B. The average score of femora with positive bacterial cultures was 3.4, of femora with negative cultures 2.1. The correlation between cultures (log CFU) and X-ray scores was 0.446. The average percentage of cement filling the femoral cavity was 34.8%.

DISCUSSION We conclude that AMP are an effective antibiotic agent in this osteomyelitis prevention model. These results show a proof of principle for the prevention of infection with peptide antibiotics released from CaP cement. Studies have shown the efficacy of numerous locally released antibiotics, AMP however, rapidly kill resistant bacteria in vitro and therefore hold great promise for future AMP based drugs. Treatment with Gentamycin was also effective, as a non-resistant bacterial strain was used. Not all rabbits with positive cultures had X-Ray changes consistent with osteomyelitis, probably due to the short follow-up period (21 days).

Studies on in vitro activity of AMP against resistant strains raise high expectations for the clinical use of these broad spectrum antimicrobials. We intend to perform animal studies to investigate the efficacy of AMP against resistant bacterial strains e.g. methicillin resistant Staph. Aureus (MRSA) and vancomycin resistant Staph. Aureus (VRSA).

REFERENCES

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