Effects of Bacteriophage Therapy on Bioburden, Biofilm, and Bone Healing in Acute Fracture-Related Infections

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INTRODUCTION:

Acute fracture-related infections are challenging based on a reported incidence of 20%, with 66% of infected patients requiring implant removal. Unfortunately, success in terms of infection resolution and fracture healing is inconsistent at best. Emerging evidence supports bacteriophage therapy to treat implant-related infections, however, associated effects on fracture healing and related complications have not been well characterized to date. Therefore, this preclinical study was designed to directly compare bacteriophage to standard treatment for acute fracture infections.

METHODS:

With IACUC approval, purpose-bred hounds (n=8; n=16 ulnas) underwent bilateral 1 cm distal ulnar defect ("fracture") creation and stabilization by plate and screw. Prior to fixation, implants were incubated in a suspension of biofilm-producing Staphylococcus aureus (OJ1) for 48 hrs. After 3 weeks, surgical sites underwent irrigation and debridement followed by 1 of 4 treatments: no additional treatment, 6 weeks of parenteral antibiotics, 7 days local bacteriophage therapy, or combination antibiotic/bacteriophage therapy. The bacteriophage cocktail was verified to be specific to OJ1 immediately prior to use. Dogs were monitored for adverse events and were humanely euthanized after 11 weeks. Quantitative microbial cultures (QMC) and radiographic assessments were performed at weeks 3 and 11. Radiographic healing was determined by calculating the area (mm2) of remaining ulnar defect at each timepoint. Ulnas were recovered and assessed for callous formation/maturity, biofilm, and bacterial load using semi-quantitative histomorphometry. Groups were compared for statistically significant ($p < 0.05$) differences using one-way ANOVA.

RESULTS:

At 3 weeks, all fracture sites had clinically and microbiologically confirmed surgical site infections. All surgical wounds remained intact and no adverse events were noted. When comparing QMCs at week 11, all treatments were superior to control ($p < 0.001$). Groups receiving bacteriophage therapy had significantly lower bacterial loads (545 +/- 204 CFU/g) when compared to non-bacteriophage groups (45,065 +/- 4,409 CFU/g) ($p=0.033$). Bridging callus formation (defect fill) was significantly better ($p=0.01$) for dogs receiving bacteriophage therapy compared to non-bacteriophage groups (Figure 1). Semi-quantitative histomorphometry for biofilm formation indicates that bacteriophage inclusive samples had less biofilm formation, 1.1(0.5-2) to 1.5 (0.5-2.5), $p=0.15$ (Figure 2). Histomorphometry also indicated bacteriophage inclusive samples had higher percentage of bone growth (21.9 +/- 5.6 vs. 19.4 +/- 6.9, $p=0.45$) and bone/cartilage formation (30.3 +/- 11.4 vs. 25.9 +/- 9.8, $p=0.43$) (Figure 3).

DISCUSSION AND CONCLUSION:

Based on initial data, 7 days of local bacteriophage treatment is at least *as effective as* 6 weeks of antibiotics in the treatment of acute fracture related infection when examining cfu/g. However, bacteriophages are superior for bone healing when compared to antibiotic therapy. Histomorphometry from the first set of 16 ulna samples are indicative of improved clinical characteristics, although not statistically significant. However, an additional 16 ulna sample population utilizing same methods has recently concluded. If similar results are produced, biofilm, cfu/g, and bone/cartilage formation would be statistically significant for positive effects of bacteriophages in infection prevention and fracture healing.

At the time of writing this abstract, radiographic findings and CFU/g were available for the second round of animal testing, which produced similar data as the first round of hounds. Bacterial counts and histomorphometry for the second round is pending.