Δ Treatment of Acute Fracture Related Infections Utilizing Bacteriophage Therapy

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Purpose: 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: 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 h. 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 (QMCs) and radiographic assessments were performed at weeks 3 and 11. Radiographic healing was determined by calculating the area (mm^2) of remaining ulnar defect at each time point. Ulnas were recovered and assessed for callus formation/maturity, biofilm, and bacterial load using semi-quantitative histomorphometry. Groups were compared for statistically significant ($P < 0.05$) differences using 1-way analysis of variance.

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 ($P < 0.001$) and bacteriophage groups had lower bacterial loads when compared to antibiotics alone, however, this did not reach statistical significance (Table). However, bridging callus formation (defect fill) was significantly better ($P = 0.01$) for dogs receiving bacteriophage therapy compared to antibiotics alone (Table). 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), $P = 0.15$. 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, $P = 0.43$). However, these did not reach significance.

Conclusion: Based on initial data, 7 days of local bacteriophage treatment is as effective as antibiotics in the treatment of acute fracture-related infection when examining colony-forming units/g. However, bacteriophages are superior for bone healing when compared to antibiotic therapy. Histomorphometry and complete data on 16 additional ulnas will be available for presentation at the OTA Annual Meeting.

Table

	3 wk Defect Fill (mm^2)	11 wk Defect Fill (mm^2)	3 wk CFU/g	11 wk CFU/g
Control	44.4 ± 9	75.6 ± 16	$1.9x10^4$ ± 2264	178,098 ± 13521
AB	45.4 ± 8	61.6 ± 12	$1.4x10^4$ ± 3133	1,195 ± 789
Phage	52.9 ± 7	88.7 ± 8	$1.7x10^4$ ± 2965	969 ± 490
AB-Phage	53.6 ± 11	85.2 ± 11	$1.3x10^4$ ± 3898	369 ± 266
3 week defect fill: all groups ANOVA, NSD p=0.31				
11 week defect fill: combination therapy sig > abx, ANOVA, **p=0.01**				
11 week defect fill: bacteriophage therapy sig > abx, ANOVA, **p=0.01**				
3 week CFU/g: comparing all 4, ANOVA, NSD, p=0.87				
11 week CFU/g: AB, phage, combo all sig < control, ANOVA, **p<0.001**				

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