Bacteriophage Therapy for Treatment of Fracture-Related Infections in a Preclinical Canine Model

Kyle Schweser, Chantelle C. Bozynski, Aaron M. Stoker, Tamara Gull, James L. Cook
Thompson Laboratory for Regenerative Orthopaedics, University of Missouri, Columbia, MO
cookjl@health.missouri.edu

Disclosures: Kyle Schweser (1-ODI; 3B-Johnson & Johnson, Carbofix; 5-Arthrex), Chantelle C. Bozynski (N), Aaron M. Stoker (1-MTF), Tamara Gull (N), James L. Cook (1-Arthrex, MTF; 2-Arthrex; 3B- Arthrex, Bioventus, Collagen Matrix Inc, Trupanion; 5-Arthrex, AO Trauma, Collagen Matrix Inc, Cellularity, MTF, NIH, Organogenesis, Purina, Regenosine, SITES Medical; 7B-Thieme; 8-J of Knee Surgery; 9-MTN, MTF)

INTRODUCTION: Fracture-related infections occur in up to 20% of patients treated with internal fixation. With current standard-of-care management, success in terms of infection resolution and fracture healing is inconsistent at best and the financial burden on patients and hospital systems remains high. 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. This preclinical study was designed to directly compare bacteriophage to standard-of-care treatment for management of acute fracture-related infections in a canine model. We hypothesized that bacteriophage treatment would be superior to standard-of-care antibiotic therapy in reducing bacterial load, promoting bone healing, and mitigating biofilm production on implants in acute fracture-related infections.

METHODS: With IACUC approval, purpose-bred hounds (n=16; n=32 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 (**Control**), 6 weeks of parenteral sulfamethoxazole/trimethoprim (480 mg) BID, to which the *Staphylococcus aureus* strain demonstrated susceptibility *in vitro* (**Abx**), 7 days local bacteriophage therapy (**Phage**), or combination antibiotic/bacteriophage therapy (**Abx-Phage**). 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 (mm^2) of remaining ulnar defect at each time point utilizing imaging software (ImageJ, FIJI). 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 t-tests, rank sum tests, or one-way ANOVA.

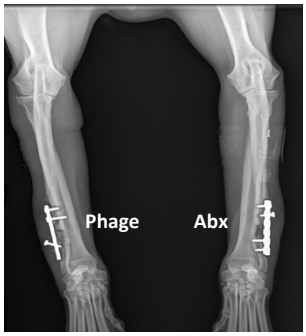

Figure – Endpoint Radiographs

RESULTS (Figure and Table): At 3 weeks (pre-treatment), all fracture sites had confirmed surgical site infections based on clinical and microbial culture assessments. All surgical wounds remained intact with varying degrees of drainage, allowing implants to be retained. When comparing QMCs at week 11 (8 weeks post-treatment endpoint), all treatment groups were superior to controls ($p < 0.001$) in terms of reducing bacterial loads and bacteriophage groups had lower bacterial loads when compared to antibiotics alone, however, this latter difference did not reach statistical significance. Bridging callus formation (defect fill) was significantly more complete ($p=0.01$) for dogs receiving bacteriophage therapy compared to antibiotics alone. Semi-quantitative histomorphometry for biofilm formation indicated that implants in bacteriophage-treated fractures were associated with less biofilm formation and that bacteriophage-treated fractures were associated with higher percentage of bone and bone/cartilage formation in the fracture gap when compared to other groups. However, these differences did not reach statistical significance.

DISCUSSION: This study represents one of the first to examine the effects of bacteriophage therapy on acute fracture related infections and bone healing. Based on initial data, 7 days of local bacteriophage treatment was as effective as 6 weeks of parenteral antibiotics in the treatment of acute fracture-related infection when examining bacterial load in a preclinical canine model. However, bacteriophage treatment was superior in promoting radiographic bone healing when compared to antibiotic therapy in this model. In addition, bacteriophage treatment was associated with subjectively less biofilm production on implants and more bone and cartilage formation in the fracture gap based on initial assessments.

SIGNIFICANCE/CLINICAL RELEVANCE: These promising results provide evidence for a treatment option for acute fracture-related infections that may be safer and more effective, as well as potentially more cost-effective, than the current standard-of-care protocol. Ongoing research is aimed at optimizing dosing regimens toward implementation in human clinical trials.

Table – Data Summary

	Pre-Treatment XR Fill (%)	Endpoint XR Fill (%)	Pre-Treatment QMC (CFU/g)	Endpoint QMC (CFU/g)		Endpoint Biofilm Score	Endpoint Bone (%)	Endpoint Bone+ Cartilage (%)
Control	46.9 ± 12	46.6 ± 13	51,972 ± 10,858	88,589 ± 24,950	**No Phage**	1.5 (0.5-2.5)	19.4 ± 6.9	25.9 ± 9.8
Abx	47.9 ± 7	58.4 ± 12	34,876 ± 9,692	1,195 ± 789				
Phage	48.4 ± 13	76.7 ± 10	39,731 ± 12,327	969 ± 490	**Phage**	1.1 (0.5-2)	21.9 ± 5.6	30.3 ± 11.4
Abx-Phage	43.4 ± 10	78.7 ± 7	57,632 ± 15,060	369 ± 266				