Assessment of Bacteriophage Pharmacokinetic Parameters after Intraarticular Delivery in a Rat Prosthetic Joint Infection Model

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INTRODUCTION:

Bacteriophage (phage) therapy has garnered interest as a potential adjunct for prosthetic joint infection (PJI) eradication, as they are well tolerated *in vivo,* highly targeted, and many are able to penetrate and disrupt biofilms. However, little is known about optimal phage dosing, and basic pharmacokinetic parameters remain unknown. Consequently, we performed a pharmacokinetic assessment of phage therapy for PJIs using a *S. epidermidis* (*S. epi*) rat PJI model. Our goal was to generate a set of pharmacokinetic parameters to better inform phage therapy investigations and clinical dosing for PJIs.

METHODS:

For 52 male Sprague Dawley rats (aged 12-14 weeks), we established a prosthesis model via a trans-articular placement of 0.8mm Kirschner wires into the right distal femur using previously described techniques.

For our PJI model (n=27 rats), we used *S. epi* ATCC 35984 (ATCC, Manassas, VA), a known biofilm forming, ampicillin-sensitive strain, for inoculation. We employed an obligate lytic bacteriophage vB_SepM_Alex (Leibniz Institute-DSMZ, Braunschweig-Süd, Germany), for which we previously demonstrated ATCC 35984 susceptibility. An intraarticular inoculum of ~10^6 colony forming units (CFU) of *S. epi* was administered under fluoroscopic guidance immediately following prosthesis insertion. Sterile controls received no inoculum.

For phage delivery, ~10^8 plaque forming units (PFU) of vB_SepM_Alex (or saline for controls) was percutaneously injected into the knee under fluoroscopic guidance 5 days postoperatively. Rats were euthanized between 30 minutes and 48 hours post phage-injection. Periarticular tissue was harvested and assessed for mean CFUs and PFUs after homogenization and plate incubation. Blood and implant sonication fluid were similarly assessed.

All animal-based procedures were conducted in accordance with the Institutional Animal Care and Use Committee. Pharmacokinetic modelling was performed using the PKSolver plug-in for Microsoft Excel (Microsoft, Redmond, WA). Alpha was set at 0.05.

RESULTS:

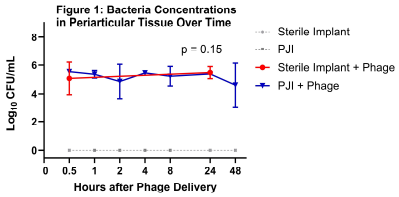
All PJI rats demonstrated infection establishment in periarticular tissues (27/27) based on plated culture. Mean CFUs in the PJI rats receiving phage therapy ranged from 5.2-5.5 $\log_{10}$ CFU/mL (**Figure 1**). Bacterial concentrations were not significantly different at 0.5 and 24 hrs between PJI groups receiving phage versus saline control (Wilcoxon Rank Sum Test, Z=-1.43, p=0.15). Maximum mean phage concentrations were recorded at the first timepoint of 0.5 hours (5.3 $\log_{10}$ PFU/mL in the PJI group and 5.4 $\log_{10}$ PFU/mL for sterile controls) (**Figure 2**). Measured phage concentrations over time between the sterile and PJI groups were not significantly different (Wilcoxon Rank Sum Test, Z=-0.15, p=0.88). No phages or bacteria could be isolated from blood. Phage and bacteria isolated from sonicated fluid were near the limit of detection of our assays and were not further analyzed.

We performed a noncompartmental pharmacokinetic analysis of delivered phage in periarticular tissues for our sterile implant group, PJI group, and a combined analysis (**Table 1**). Estimated half-lives were under 6 hours (Combined: 3.73 [IQR, 1.45, 10.07], Sterile Group: 5.59 [3.45, 14.57], PJI Group: 1.87 [1.01, 6.72]). Maximum phage concentrations were reached within 2 hours after administration (Combined: 0.75 [0.50, 1.75], Sterile Group: 1.00 [0.75, 1.50], PJI Group: 0.50 [0.50, 1.25]). Estimated mean residence time of phages prior to clearance were approximately 3 hours (Combined: 3.04 [1.44, 4.19], Sterile Group: 3.06 [1.99, 17.09], PJI Group: 3.02 [1.78, 3.79]).

DISCUSSION AND CONCLUSION:

To the authors' knowledge, this study represents the first to assess pharmacokinetics of bacteriophages in an animal model applied in the setting of prosthetic joint infection. We identified basic pharmacokinetic parameters for intra-articularly applied phages in the setting of PJI which can inform future *in vivo* dosing studies as well as clinical applications. Our findings suggest relatively short phage persistence *in vivo*, supporting emerging evidence that repeated

or continuous phage dosing may be more efficacious for bacterial reduction. Future studies should consider replicating pharmacokinetic assessments across different bacteria-phage pairs and include larger animal models to enable repeated intraarticular sampling within individual animals over time.



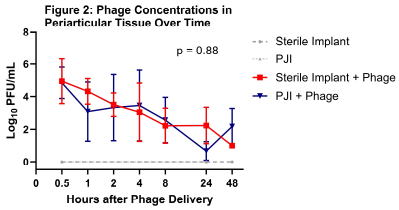


Table 1: Pharmacokinetic Parameters of Bacteriophage vB_SepM_Alex Following Intraarticular Injection (~10^8 PFU)

Parameter	Unit	Combined Pharmacokinetic Assessment (median [IQR]) (n=42)	Sterile Implant + Phage (median [IQR]) (n=21)	PJI + Phage (median [IQR]) (n=21)
λz	1/hr	0.25 [0.08, 0.49]	0.12 [0.08, 0.33]	0.37 [0.22, 2.63]
$t_{1/2}$	hr	3.73 [1.45, 10.07]	5.59 [3.45, 14.57]	1.87 [1.01, 6.72]
t_{max}	hr	0.75 [0.50, 1.75]	1.00 [0.75, 1.50]	0.50 [0.50, 1.25]
C_{max}	PFU/mL	1.61 x10^5 [2.75 x10^4, 2.83 x10^5]	2.05 x10^4 [1.48 x10^4, 3.94 x10^5]	2.73 x10^5 [1.61 x10^5, 2.80 x10^5]
$AUC_{0\text{-}t}$	PFU/mL *hr	1.27 x10^5 [7.79 x10^4, 3.77 x10^5]	6.95 x10^4 [5.09 x10^4, 2.61 x10^5]	1.50 x10^5 [1.27 x10^5, 7.69 x10^5]
$AUC_{0\text{-}\infty}$	PFU/mL *hr	1.31 x10^5 [8.06 x10^4, 3.78 x10^5]	7.01 x10^4 [6.54 x10^4, 2.62 x10^5]	1.50 x10^5 [1.31 x10^5, 7.75 x10^5]
$AUMC_{0\text{-}\infty}$	PFU/mL *hr^2	3.75 x10^5 [2.46 x10^5, 1.52 x10^6]	4.12 x10^5 [3.13 x10^5, 1.15 x10^6]	3.39 x10^5 [2.10 x10^5, 3.36 x10^6]
$MRT_{0\text{-}\infty}$	hr	3.04 [1.44, 4.19]	3.06 [1.99, 17.09]	3.02 [1.78, 3.79]
Vz/F_{obs}	(PFU)/(PFU/mL)	2.09 x10^3 [1.34 x10^3, 2.61 x10^3]	2.68 x10^3 [2.23 x10^3, 2.93 x10^4]	1.19 x10^3 [6.64 x10^2, 1.80 x10^3]
Cl/F_{obs}	(PFU)/(PFU/mL)/hr	7.79 x10^2 [3.32 x10^2, 1.29 x10^3]	1.43 x10^3 [8.23 x10^2, 1.54 x10^3]	6.66 x10^2 [3.68 x10^2, 7.79 x10^2]

λz: terminal elimination rate constant estimate. $t_{1/2}$: half-life estimate. t_{max}: time of maximum plasma concentration. C_{max}: maximum plasma concentration estimate. $AUC_{0\text{-}t}$: Area under the concentration-time curve (total exposure estimate) from zero to last measured timepoint (48 hrs). $AUC_{0\text{-}\infty}$: Area under concentration-time curve (total exposure estimate) over time. $AUMC_{0\text{-}\infty}$: area under the moment curve, from zero to infinity. $MRT_{0\text{-}\infty}$: estimate of mean residence time. Vz/F_{obs}: apparent volume of distribution. Cl/F_{obs}: apparent total plasma clearance.