Efficacy of Bacteriophages in a *Staphylococcus aureus* Nondiabetic or Diabetic Foot Infection Murine Model

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ABSTRACT This study investigated the *in vivo* efficacy of three bacteriophages combined compared with linezolid in two mouse models (nondiabetic and diabetic) of *Staphylococcus aureus* foot infection. In both models, a single injection of bacteriophages in the hindpaw showed significant antibacterial efficacy. Linezolid was as effective as bacteriophages in nondiabetic animals but ineffective in diabetic animals. These findings further support preclinical and clinical studies for the development of phage therapy.

KEYWORDS *Staphylococcus aureus*, bacteriophages, diabetic foot infection, preclinical drug studies

Diabetic foot infections are common and frequently polymicrobial, but *Staphylococcus aureus* remains the most frequently isolated pathogen (1–3). The emergence of antibiotic resistance and clinical failures requires the development and the evaluation of innovative alternatives against such infections. In this context, phage therapy is being reexamined as a complementary approach to antibiotics in clinical settings.

However, even though the potency of phage therapy was recently demonstrated in experimental models (4, 5), little is known about its *in vivo* success in this indication of diabetic foot infection. The assembly of the three bacteriophages described in the study has not been examined in a clinical trial, although it has already been successfully tested as an experimental treatment for compassionate use (6, 7).

The current study aimed to evaluate the pharmacokinetics of three lytic bacteriophages in treatment-naive mice and their therapeutic potential in the treatment of an acute experimental methicillin-susceptible *S. aureus* (MSSA) nondiabetic or diabetic foot infection model.

The clinical MSSA Hocil17 strain was used. Three anti-*S. aureus* purified bacteriophages were provided by Pherecydes Pharma (6).

Immunocompetent BALB/c female mice, weighing 20 to 25 g, were used (Charles River). The experimental protocol was approved by the ethics committee (APAFIS nos. 11757 and 11242) and performed in accordance with European Institute of Health EU directive 86/609.

For the pharmacokinetic study, animals received 1×10^8 or 1×10^9 PFU per phage locally (intra-articularly at the patella or in the hindpaw) or intravenously. Groups of five animals were sacrificed at 1, 2, 6, 24, 48, and 72 h postinjection. Blood, spleen, kidney,

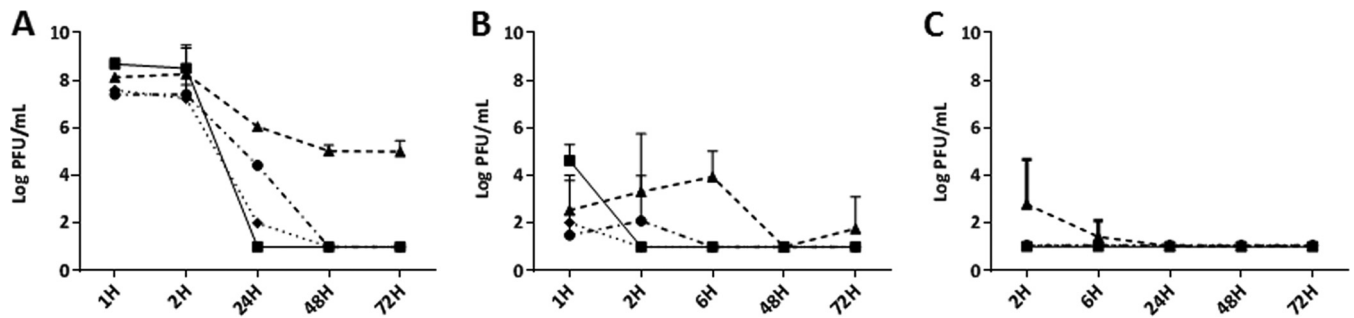Address correspondence to D. Croisier, delphine.croisier@vivexia.fr.

FIG 1 Pharmacokinetics of an assembly of three bacteriophages after systemic injection (A) of 1×10^9 PFU/mouse or intraarticular injection at the kneecap (B) or in the hindpaw (C) of 10^8 PFU/mouse. Representation of mean $\pm$ SD ($n = 5$) PFU/ml in different organs or biological fluid at 1, 2, 6, 24, 48, and 72 h. ■, blood; ▲, spleen; ●, kidney; and ◆, liver. Detection threshold, 10 PFU/ml.

and liver were collected. The bacteriophage titers (PFU/ml) were determined by a validated spotting method.

Diabetes was induced in 80 mice by two intraperitoneal (i.p.) injections of 150 mg/kg streptozotocin (VWR) at 48 h apart. Two weeks after induction, blood glucose levels were recorded (glucometer Contour Next One) (8). Animals with insufficient levels (<300 mg/dl) before infection were excluded.

Nondiabetic ($n = 80$) and diabetic ($n = 60$) mice were randomized to different treatment groups. Mice were anesthetized and infected with a bacterial inoculum of 1×10^7 CFU/mouse at a depth of 2 to 4 mm into the planter proximal aspect of the hindpaw (4).

Thirty minutes postinfection, animals received a single dose of linezolid 25 mg/kg i.p., bacteriophages at 1×10^7 or 1×10^8 PFU/phage into the hindpaw, or the assembly of bacteriophages plus linezolid. The control animals were given vehicle. Bacterial counts were assessed on days 1, 2, 3, and 4.

Treatments were compared by one-way analysis of variance followed by a *post hoc* Bonferroni test (GraphPad Prism 7.0).

According to the pharmacokinetic study, a high concentration of bacteriophages was present in all tested organs 2 h after the intravenous injection (Fig. 1A). Bacteriophages were still detectable at 72 h in the spleen, whereas they rapidly decreased in blood, liver, and kidney to become undetectable at 48 h.

After local administration, phage titers in the organs and blood were low compared with those after systemic injection (Fig. 1B and C). With intra-articular administration, a positive bacteriophage titer was observed in the blood and organs 1 h postinjection; the bacteriophages then rapidly disappeared after 2 h in blood and liver. In comparison, positive bacteriophage titers were recorded in the kidney and spleen, respectively, at 2 and 6 h postinjection, before decreasing to undetectable levels at 6 and 48 h. With hindpaw administration, no phage was recovered in the organs, except in the spleen at 2 h postinjection, but this signal completely disappeared at 6 h.

Overall, no clinical signs of toxicity (mortality, loss of weight, reduced activity) were observed during the pharmacokinetic study.

In nondiabetic controls, the bacterial load was stable over 3 days and started to decline on day 4 (Fig. 2). From day 1, regardless of dosage used, a single injection of the bacteriophage assembly was as effective as linezolid in nondiabetic infected animals. No antibacterial synergistic effect was observed in mice receiving the concomitant therapy (phages plus linezolid).

The bacterial load in untreated diabetic mice remained stable over 4 days (Fig. 3), thus longer than in nondiabetic mice. A single injection of bacteriophages at 10 MOI (multiplicity of infection) was significantly effective throughout the study. Surprisingly, linezolid was ineffective in treating diabetic mice. No antibacterial synergistic effect was recorded in diabetic mice receiving the concomitant therapy (phages plus linezolid).

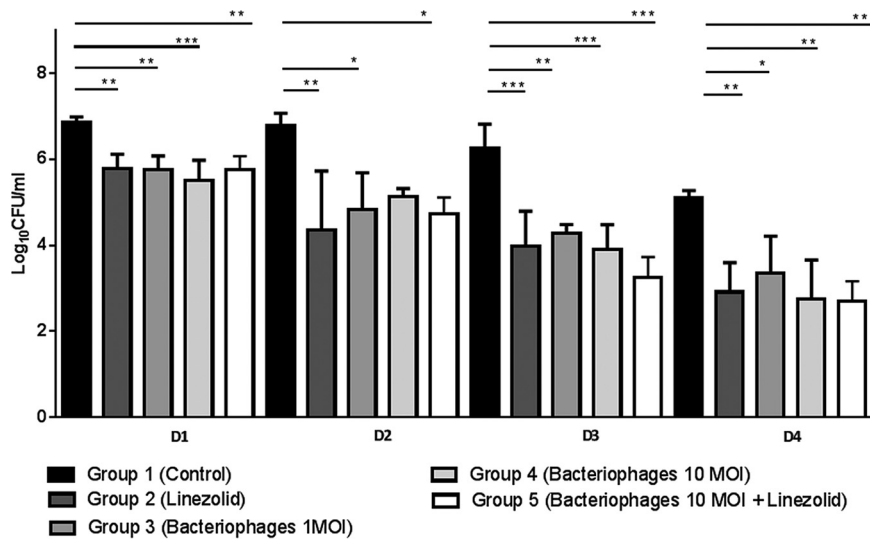The data set detailed in this proof-of-concept preclinical study demonstrates that a

FIG 2 Bacterial load ($\log_{10}$ CFU/ml), in hindpaws of nondiabetic BALB/c mice ($n = 4$/group and time point) after treatment with an assembly of three bacteriophages (1 or 10 MOI), linezolid (25 mg/kg i.p.), and the combination of phages plus linezolid. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

single injection of three combined phages at the infection site was as effective as a single systemic injection of linezolid in reducing bacterial burden in the hindpaw of nondiabetic mice. Of note, in the diabetic model, the bacteriophage assembly was more active than linezolid, which failed to resolve the infection. Despite being the recommended treatment in the guidelines, linezolid has been associated to clinical failures (9, 10, 11).

In both models, no antibacterial synergistic effect was demonstrated in mice receiving the concomitant therapy (bacteriophages plus linezolid). Interestingly, the assembly of three lytic bacteriophages administered systemically or locally at high concentrations was very well tolerated and cleared rapidly from the bodies of noninfected mice.

These encouraging but preliminary findings suggest that bacteriophages may represent an interesting alternative for the treatment of such acute *S. aureus* infections. However, their potential requires further investigation in other relevant *in vivo* models, including biofilm-associated infections (e.g., chronic diabetic foot ulcer, orthopedic
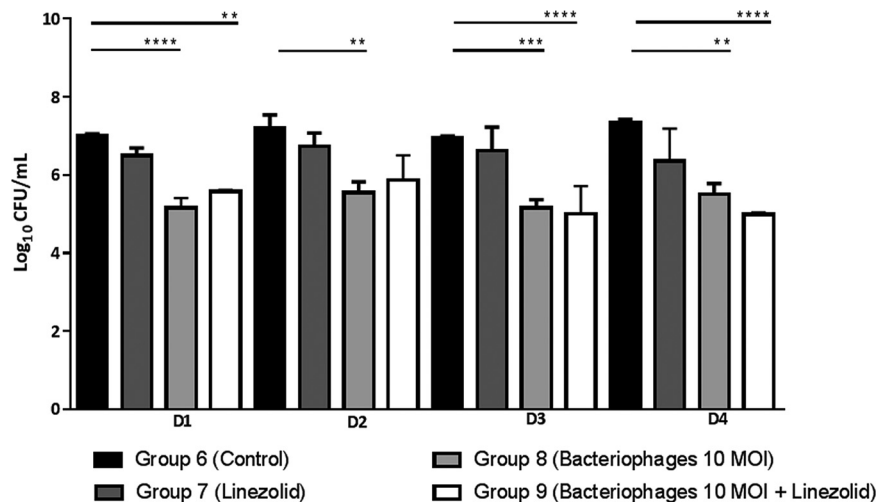

FIG 3 Bacterial load ($\log_{10}$ CFU/ml) in hindpaws of diabetic BALB/c mice ($n = 3$ or 4/group and time point) after treatment with an assembly of three bacteriophages, linezolid (25 mg/kg oral), and the combination of phages plus linezolid. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

implant-associated infection models) to better define the most accurate administration parameters for future clinical trials. Even if the *in vitro* activity of this assembly of bacteriophages is found to be comparable in multidrug-resistant (MDR) or non-MDR *S. aureus* strains (data not shown), its potential needs to be assessed in MDR-induced preclinical models as well.

Finally, this assembly of bacteriophages has entered clinical development. It is currently being investigated in two trials, one involving diabetic foot ulcer (https:// clinicaltrials.gov/ct2/show/NCT02664740) and the other involving prosthetic joint infection indications (http://www.infectiologie.com/UserFiles/File/formation/desc/2016/ seminaire-octobre-2016/phagottt-16-09-pr-michel-dupon.pdf), to determine whether this activity translates into comparable broad clinical efficacy.

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