



Dynamical Modeling of Phage Therapy using *in vitro/in vivo* preclinical data

Dongwoo Chae¹, Jun Seok Cha¹, Kyungnam Kim^{2,3}, Yeajune Cho^{2,3}, Ye Jeong Lee^{2,3}, Rynha Chang^{2,3},

Yoonyoung Jang^{2,3}, Yeohun Hyun^{2,3}, Keon Hee Lee^{2,3}, and Dongeun Yong^{2,3}

¹Department of Pharmacology, Yonsei University College of Medicine, Seoul, Korea.

²Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul, Korea.

³Microbiotix Corporation, Seoul, Korea.

OBJECTIVES

The emergence of multi-drug resistant (MDR) pathogens is one of the greatest threats to global health. The pipeline of new antibacterial drugs in clinical development is inadequate and there is a need for alternate therapeutics. Phage therapy is increasingly recognized as a potential candidate to combat antimicrobial resistance, but among many limitations is its poor characterization of pharmacokinetic-pharmacodynamic (PKPD) properties. Here, we applied dynamical modeling to better understand the efficacy of phage therapy targeting carbapenem-resistant *P. aeruginosa* based on *in vitro* and *in vivo* preclinical data.

METHODS

1. Data Acquisition

Three phage strains – 1304a, 1172, and 1787 – were used to generate preclinical data characterizing phage adsorption rate, one-step growth curve, and lysis kinetics. Three different types of *in vitro* experiments were conducted – lysis test, one-step growth test, and adsorption test. *In vivo* dose range finding experiments were performed with a total of 48 mice and 48 rats. 4 dose groups of 12 animals were intratracheally inoculated with *P. aeruginosa* 15-4, and 4 from each group were randomly chosen to administer phage 1304a intravenously at a multiplicity of infection (MOI) of 50.

2. Model Building

1) Bacterial growth / Phage infection model

The following system of ordinary differential equations describes the dynamics of bacteria-phage interactions.

$$\frac{dB_1}{dt} = gB_1(1 - B_{tot}/B_{max,1}) - k_i P_i B_1 \quad (1)$$

$$\frac{dB_2}{dt} = gB_2(1 - B_{tot}/B_{max,2}) - k_i P_i B_2 \quad (2)$$

$$\frac{dP_i}{dt} = -k_i P_i (B_1 + B_2) + bw(L_1 + L_2) \quad (3)$$

$$\frac{dI_1}{dt} = -k_i P_i B_1 - vI_1 \quad (4)$$

$$\frac{dL_1}{dt} = vI_1 - wL_1 \quad (5)$$

$$\frac{dI_2}{dt} = -k_i P_i B_2 - vI_2 \quad (6)$$

$$\frac{dL_2}{dt} = vI_2 - wL_2 \quad (7)$$

$$\frac{dR}{dt} = g'R(1 - B_{tot}/R_{max}) \quad (8)$$

$$B_{tot} = B_1 + B_2 + I_1 + I_2 + L_1 + L_2 + R \quad (9)$$

(k_i : adsorption rate of phage strain i , v : phage replication rate, w : phage lysis rate, b : burst size, g : growth rate of phage-susceptible bacteria, g' : growth rate of phage-resistant bacteria, B_{max} : carrying capacity of phage-susceptible bacteria, R_{max} : carrying capacity of phage-resistant bacteria)

In the above, B_1 and B_2 denote bacterial subpopulations with different carrying capacities. P_i denotes phage titer, with subscript i used to represent different phage strains. Two transit compartments – I and L – were introduced to mimic the latent period between phage infection and host cell lysis.

2) Phage adsorption kinetics

$$\frac{dP}{dt} = -kPB_0 + k'C \quad (10)$$

$$\frac{dC}{dt} = kPB_0 - (k' + k'')C \quad (11)$$

(k : rate of reversible binding, k' : rate of reversible dissociation, k'' : rate of irreversible binding)

The above equations were used to fit the phage adsorption curves that were generated with bacterial density of 1.0×10^8 CFU/mL mixed with phages 1304a and 1172 given as multiplicity of infection (MOI) of 0.1 and 0.01, respectively. P and C denote free and reversibly bound phage particles.

3) Survival modeling

Rat and mice survival data treated with phage 1304a were linked with the bacteria-phage interaction model. Mortality hazard was assumed as being proportional to the total bacterial burden. Constant, Weibull, and log-logistic hazard functions were tested to describe the time-varying hazard.

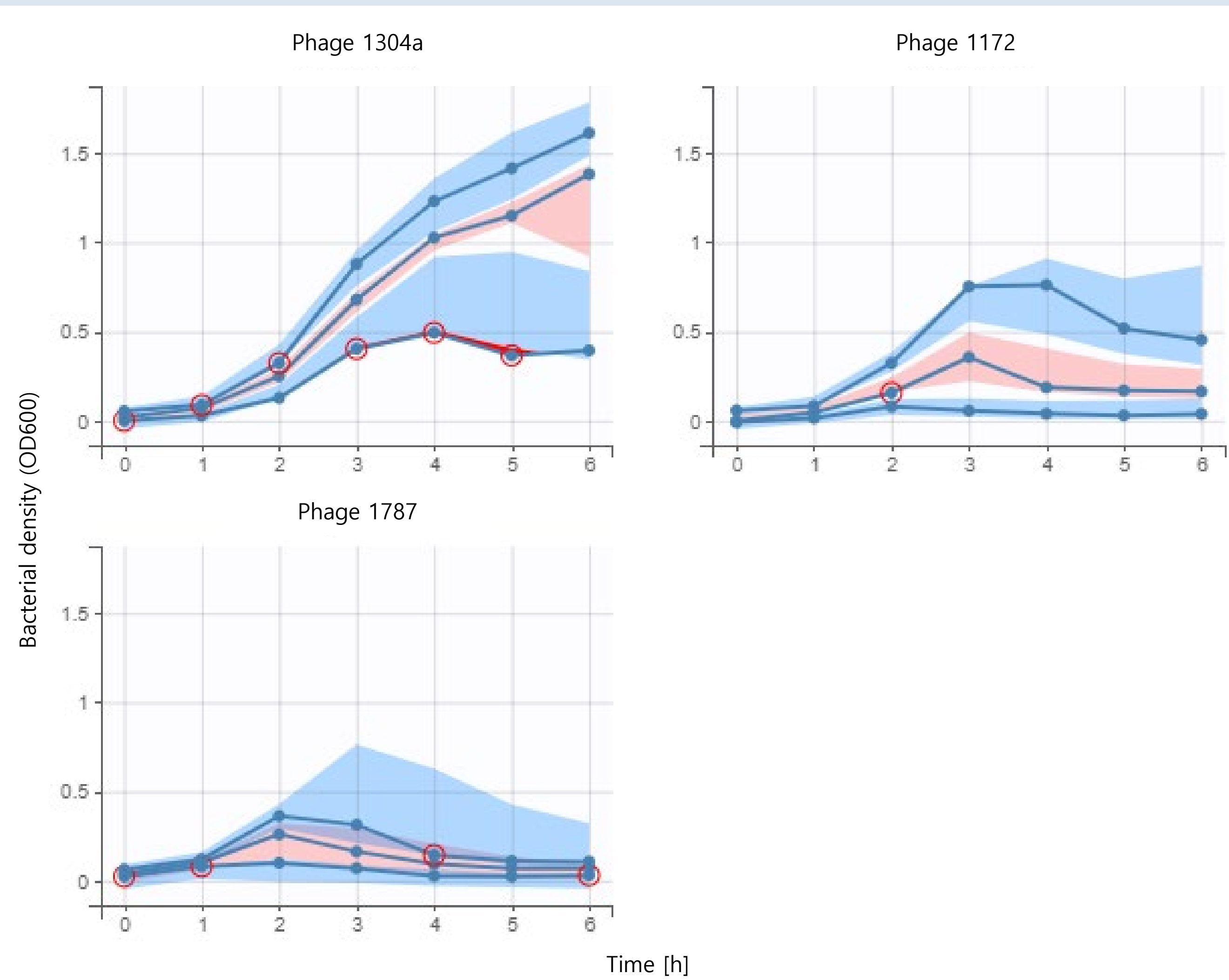


Figure 1. Visual Predictive Check of the *in vitro* bacterial growth / phage infection model
(Red line: 2.5%, 50%, and 97.5% of observations, Red shaded area: 90% CI of 50% of population predicted values, Blue shaded area: 90% CI of 2.5% and 97.5% of population predicted values)

RESULTS

Table 1. Parameter estimates of the bacterial growth / phage infection model

Parameter	Estimate	SE	RSE(%)
Initial bacteria OD600			
Experiment 1	0.017	0.0012	7.26%
Experiment 2	0.008	0.0019	23.58%
B_1/B_2 growth rate constant (g, /h)	1.66	0.029	1.73%
R growth rate constant (g', /h)			
1304a	1.65	0.025	1.54%
1172	1.17	0.0031	0.27%
1787	0.0	-	-
Fraction of B_2	0.042	0.0013	3.06%
Carrying capacity (OD600)			
B_1	2.62	0.021	0.809%
B_2	1.07	0.0016	0.147%
R	1.8	0.011	0.59%
Adsorption rate constant (ml/h/OD600)			
1304a	0.38	0.14	35.9%
1172	6	1.56	26.1%
1787	12.63	0.12	0.935%
Fraction of resistant bacteria, f_R			
1304a/1172	0.0091	0.0051	56.5%
1787	0.0	-	-
v (/h)	0.39	0.042	11.0%
w (/h)	11.29	0.087	0.773%
b	124.2	13.01	10.5%

Table 2. Parameter estimates of the phage adsorption model

Parameter	Estimate	SE	RSE(%)
k ($\times 10^{-8}$ ml/min)	1.38	0.036	2.61%
k' (/min)			
1304a	1.79	0.094	5.27%
1172	0.16	0.016	10.0%
k'' (/min)	0.24	0.013	5.46%

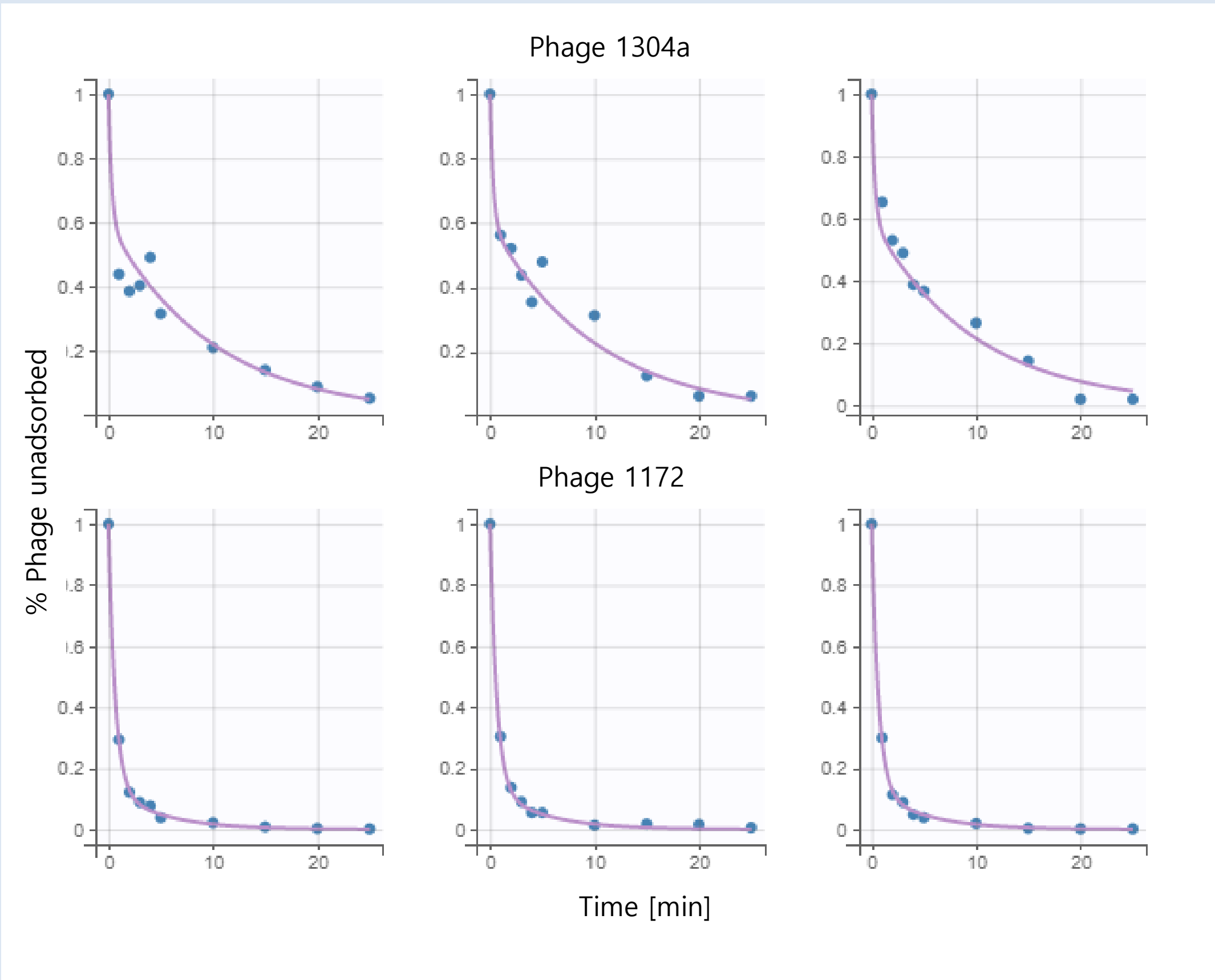


Figure 2. Predicted adsorption curves (violet line) superimposed on observed values (blue dots)

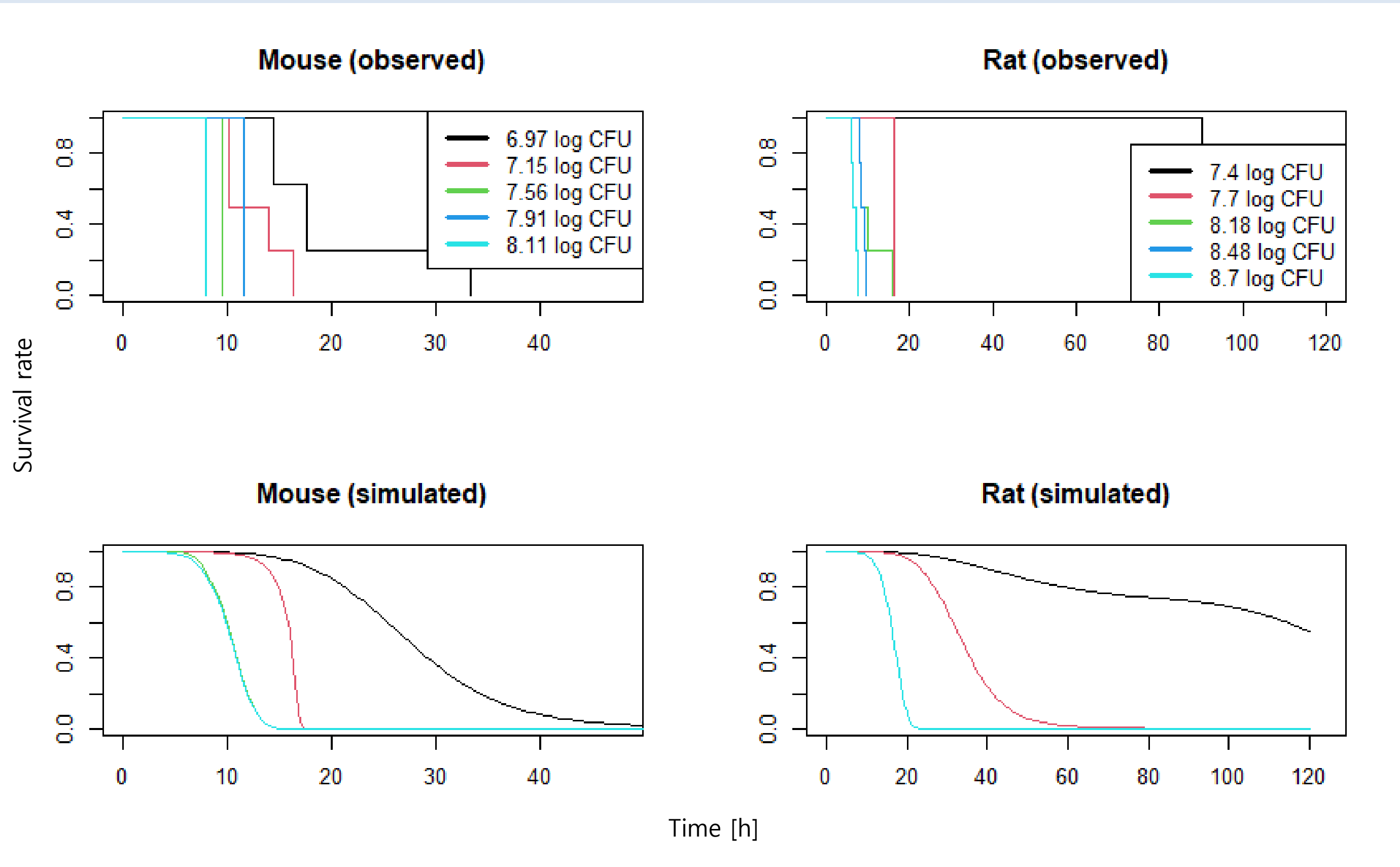


Figure 3. Mirror plots of observed and simulated survival curves given different doses of *P. aeruginosa* treated with phage 1304a (MOI=50)

CONCLUSIONS

This study successfully applied modeling and simulation methodologies to characterize, understand, and aid in the subsequent development of the phage therapy product. *In vitro* phage properties well predicted the *in vivo* efficacy, whereby low adsorption rate constant translated to poor efficacy. Our work is expected to serve as a good reference for future modeling applications directed at developing successful phage therapy.

REFERENCES

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