Comparison of Three Aqueous Aerosol Inhalation Devices for Delivering Anti-Tuberculosis Bacteriophage D29

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INTRODUCTION

Bacteriophages (phages) are viruses that typically infect a narrow spectrum of bacteria [1]. Clinical treatment using pulmonary phage delivery is done in parts of Eastern Europe and although literature is scarce, high efficacy has been reported [1,2]. Recently, an *in vivo* mouse study demonstrated success of aerosol phage therapy in combatting antibiotic-resistant lung infections when a sufficiently high titer (number of infectious phage) is delivered relative to the bacterial count [3]. High titer delivery requires an efficient inhalation device that does not deactivate the phage. It is therefore of interest to compare phage deactivation for different inhalation devices. While a jet

nebulizer and vibrating membrane nebulizer have previously been shown to successfully deliver phages active against other bacteria [4], here we explore their ability to deliver phage D29, which is of particular interest as D29 can effectively kill a range of mycobacteria, including *Mycobacterium tuberculosis* [5]. The ability of the Respimat soft mist inhaler to deliver phage has not been previously explored to our knowledge, and is examined here.

MATERIALS AND METHODS

Three inhalation devices were tested: 1) Pari LC Sprint jet nebulizer with Pari Boy SX Compressor (Pari GmbH, Starnberg, Germany), 2) Respimat Soft Mist Inhaler (Boehringer Ingelheim Ltd., Burlington, Canada), and 3) Aerogen Solo vibrating mesh nebulizer (Aerogen Ltd., Dangan, Ireland).

Phage D29 (Figure 1) was isolated, amplified, and purified according to well-established protocols [6].

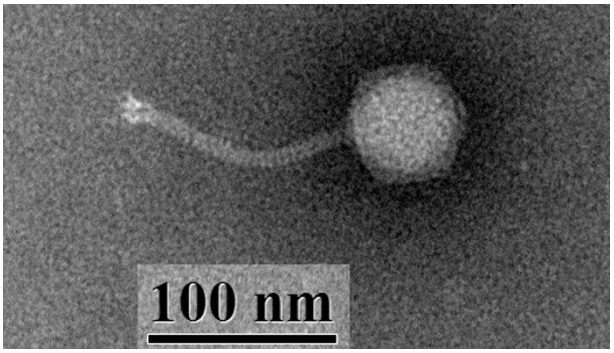

Figure 1: Transmission electron micrograph of siphovirus phage D29.

The amplified phage lysate was diluted 1:100 in isotonic saline prior to use. Plaque assays, which used the surrogate *M. smegmatis* strain mc^{2}155 to determine the number of plaque-forming units (pfu) on a plate, showed that this dilution step did not lead to

detectable phage deactivation. Stability of the saline phage preparation was observed for 18 hours at room temperature. The saline phage preparation was input to the inhalation device and a Suregard Bacterial/Viral Respiratory Filter (BIRD Healthcare, Port Melbourne, Australia) attached to the device mouthpiece. Aerosolized phage droplets captured on the filter were drawn for plaque assay to determine the output titer, which was used to calculate the percent deactivation due to aerosolization as: Deactivation = (1 - Output Titer / Input Titer) * 100%. For the Respimat, the small emitted dose (measured to be 11.6 ± 1.6 µL/actuation) meant that resuspension in phage buffer (10mM $MgSO_4$, 1mM $CaCl_2$, 10mM Tris 7.5, 68.5mM NaCl) was necessary to draw liquid for plaque assay. The resuspension step did not lead to measurable phage deactivation.

Using the output titer and the measured emitted droplet rates of 0.160 ± 0.002 mL/min for the jet nebulizer and 0.364 ± 0.025 mL/min for the vibrating mesh nebulizer, the pfu/min output delivery rate from each nebulizer was calculated.

Plaque assay measurements were performed in triplicate at multiple dilution levels. Results are presented as mean ± standard deviation of all plates with ≥5 plaques. The number of plates used to calculate standard deviation ranged from 2-6. Student's t-tests were performed at a significance level of 0.05 without assuming equal variance.

RESULTS AND DISCUSSION

Table 1 summarizes the results.

Table 1: Phage D29 input titer, output titer, deactivation and delivery rate for three clinically-relevant inhalation devices.

Inhalation Device	Input Titer (pfu/mL)	Output Titer (pfu/mL)	Deactivation (%)	Delivery Rate
Pari LC Sprint (jet nebulizer)	$2.4x10^9 \pm 0.3x10^9$	$4.4x10^5 \pm 1.1x10^5$	99.981 ± 0.005	$7.1x10^4 \pm 1.7x10^4$ pfu/min
Aerogen Solo (vibrating mesh nebulizer)	$2.3x10^9 \pm 0.4x10^9$	$9.0x10^8 \pm 2.0x10^8$	60 ± 11	$3.3x10^8 \pm 0.8x10^8$ pfu/min
Respimat (soft mist inhaler)	$1.4x10^9 \pm 0.5x10^9$	$4.0x10^8 \pm 1.6x10^8$	72 ± 14	$4.6x10^6 \pm 2.0x10^6$ pfu/dose

Deactivation was significantly greater ($p<0.05$) for the jet nebulizer as compared to either the soft mist inhaler or the vibrating mesh nebulizer. Deactivation was acceptable (~$0.5\log_{10}$ pfu/mL) for both the soft mist inhaler and the vibrating mesh nebulizer, and there was no significant difference between them ($p>0.1$).

Calculations show the vibrating mesh nebulizer can deliver a given number of pfu of D29 ~5000 times faster than the jet nebulizer. A single 11.6µL actuated dose from the Respimat could deliver as many pfu of D29 as ~10 mL of formulation from the jet nebulizer (which would require ~1hr of jet nebulizer delivery). While it would take ~70 actuations with the Respimat to deliver the same pfu as the vibrating mesh nebulizer delivers in one minute, the use of more concentrated phage preparation in the Respimat may alleviate the need for multiple actuations with this device, although this has not been explored here.

The substantial titer drop with the Pari LC Sprint may be due to repeated stress and baffle impaction associated with re-nebulization, leading to large cumulative stress on the phage. Indeed, a Collison jet nebulizer with a triple-jet head re-nebulized 99.92% of

water, with the equivalent of the entire 20mL fill volume being recirculated every 6 seconds [7]. This mechanism may explain the deactivation of liposome and large molecules observed in the literature with jet nebulizers [8, 9]. It should be noted that the sensitivity of phages to the stress of jet nebulization is phage species-dependent, since other phage species readily survive jet nebulization [4].

CONCLUSIONS

The choice of inhalation device is critical to pulmonary delivery of active phage. Of the three devices tested with the present phage preparation, the Aerogen Solo vibrating mesh nebulizer is the best inhalation device choice as it delivers high pfu quickly. The Pari LC Sprint jet nebulizer deactivates D29 and is unsuitable for delivery. The Respimat soft mist inhaler, tested with phage for the first time via this study, was not relatively harmful to D29. Given the observed differences in delivery between the three different inhalation devices examined here, testing the survival of other phages with devices that use different aerosol production methods is warranted in the development of phage cocktail – inhalation device combinations.

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