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Antibacterial Efficacy of Lytic *Pseudomonas* Bacteriophage in Normal and Neutropenic Mice Models

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Recently, lytic bacteriophages (phages) have been focused on treating bacterial infectious diseases. We investigated the protective efficacy of a novel *Pseudomonas aeruginosa* phage, PA1Ø, in normal and neutropenic mice. A lethal dose of *P. aeruginosa* PAO1 was administered via the intraperitoneal route and a single dose of PA1Ø with different multiplicities of infection (MOI) was treated into infected mice. Immunocompetent mice infected with *P. aeruginosa* PAO1 were successfully protected by PA1Ø of 1 MOI, 10 MOI or 100 MOI with 80% to 100% survival rate. No viable bacteria were found in organ samples after 48 h of the phage treatment. Phage clearing patterns were different in the presence or absence of host bacteria but PA1Ø disappeared from all organs after 72 h except spleen in the presence of host bacteria. On the contrary, PA1Ø treatment could not protect neutropenic mice infected with *P. aeruginosa* PAO1 even though could extend their lives for a short time. In *in vitro* phage-neutrophil bactericidal test, a stronger bactericidal effect was observed in phage-neutrophil co-treatment than in phage single treatment without neutrophils, suggesting phage-neutrophil co-work is essential for the efficient killing of bacteria in the mouse model. In conclusion, PA1Ø can be possibly utilized in future phage therapy endeavors since it exhibited strong protective effects against virulent *P. aeruginosa* infection.

***Keywords*:** bacteriophage therapy, *Pseudomonas aeruginosa*, neutropenic mouse

Global crises of antibiotic resistance over pathogenic bacteria have emphasized the need to develop new strategies for treating multidrug resistant bacterial infections (Preisner *et al.*, 2010). Bacteriophages (phages) are bacterial viruses that grow only in live bacterial cells (Sillankorva *et al.*, 2010) and not in animal or plant cells. Possible uses of phages as antibacterial agents were studied by d'Herelle in the 1920s (Carlton, 1999). Phage therapy, however, was nearly abandoned after the discovery of antimicrobial agents in the 1940s, except in Eastern Europe (Kutateladze and Adamia, 2008; Housby and Mann, 2009; Akinyemi *et al.*, 2010). The present situation provides a strong impetus for medical researchers to consider the therapeutic use of phages as one of the attractive alternatives in place of antibiotics (Waseh *et al.*, 2010). Phages have been studied as biocontrol agents to prevent food borne infections (Goode *et al.*, 2003; O'Flynn *et al.*, 2004; Kocharunchitt *et al.*, 2009) and *in vivo* trials have been carried out against common bacterial pathogens (Biswas, 2002; Capparelli *et al.*, 2007; Watanabe *et al.*, 2007; Heo *et al.*, 2009; Capparelli *et al.*, 2010).

Pseudomonas aeruginosa is one of the most common opportunistic pathogens, causing septicemia in debilitated patients with underlying diseases such as neoplastic diseases, diabetes, burn wound, and cystic fibrosis (Hughes *et al.*, 2002). Not only is this bacterium intrinsically resistant to many antimicrobial agents (Reynolds *et al.*, 2010; Whelan *et al.*, 2010) but it has also acquired resistance against the most effective antimicrobial agents including imipenem (Zilberberg. *et al.*, 2010). Lytic phages have been studied against *Pseudomonas* infection in experimental animal models (Ahmad, 2002; Watanabe *et al.*, 2007; Heo *et al.*, 2009; Kumari *et al.*, 2009) and they may be utilized as substitutes of antimicrobial agents in future. Recently, we isolated a novel lytic phage (PA1Ø) capable of killing *P. aeruginosa* effectively in *in vitro* study. Therefore we here evaluated the *in vivo* antibacterial efficacy of this novel phage using mouse model. We also evaluated the efficacy of PA1Ø in neutropenic mice. Phage therapy had been suggested to be safe and effective in immune-compromised hosts as well as immune-competent hosts (Borysowski and Gorski, 2008), but there are not many available documents regarding the utilization of phages in neutropenic hosts. Based on the results in neutropenic mice models, we anticipated the need of phage-neutrophil co-work as a backbone of phage's bactericidal function *in vivo*. We further assessed the phage-neutrophil bactericidal activity *in vitro*.

Materials and Methods

Preparation of phage lysate and bacteria

A novel *Pseudomonas* bacteriophage (PA1Ø) was isolated from our previous study (unpublished). The phage genome sequences are available in GenBank database under accession number HM624080. Phage lysate was prepared by double agar overlay technique (Merabishvili *et al.*, 2009). In brief, *P. aeruginosa* strain PAO1, were grown in Luria Bertani (LB) broth in a shaking incubator at 37°C up to log phase, and 100 μl of log phase bacteria were mixed with phage lysate containing 5×10^6 plaque forming unit (PFU)/ml of phages. The mixtures

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were kept at room temperature for 5 min for phage adsorption into host cells before adding into 3 ml of soft agar (LB containing 0.6% agar). The mixtures of bacteria, PA1 phage, and agar were poured onto LB agar plates and plates were incubated at 37°C for overnight. Phage lysate was harvested after eluting with 4 ml SM buffer/plate by shaking for 30 min at room temperature and centrifuged at 13000 rpm for 10 min at 4°C. The supernatant was filtered using a 0.45 μm membrane filter (Sartorius Stedim Biotech GmbH, Germany) and stored at 4°C after adding 1/25 volume of chloroform. Phage titer was determined by plaque assay and mean PFU was calculated from the data obtained from three independent assays.

Bacterial inoculum for mice infection was prepared by growing *P. aeruginosa* strain PAO1 in blood agar plates at 37°C for overnight, and washed twice with sterile PBS by centrifugation at 8,000 rpm for 10 min. The bacterial pellet was re-suspended and adjusted to an OD_{600} of 1.0 corresponding to 5×10^8 colony forming unit (CFU)/ml then diluted to 10 fold in PBS for infection of mice.

Experimental animal

In vivo experiments were carried out using pathogen-free 4-5 weeks-old male ICR mice (DBL, Korea) weighing 24-26 g. All mice were housed in a clean animal facility and they were provided clean and safe food and water. The Study was approved by Animal Care Committee of Kyungpook National University School of Medicine.

Protective efficacy of PA1Ø on normal and neutropenic mice infected by *P. aeruginosa* PAO1 strain

The minimum lethal dose of *P. aeruginosa* strain PAO1 was determined before carrying protective efficacy experiments by injecting different doses of *P. aeruginosa* strain PAO1 (5×10^4, 5×10^5, 5×10^6, and 5×10^7 CFU/mouse) in normal and in neutropenic (5×10^3, 5×10^4, and 5×10^5 CFU/mouse) mice intraperitoneally (i.p.). Five mice were utilized for each dose group.

For the evaluation of protective efficacy of PA1Ø in normal mice, three independent experiments consisting of five groups (five mice in each group) were carried out. One hundred microliters of *P. aeruginosa* PAO1 (5×10^6 CFU) and single dose of PA1Ø containing either 1 multiplicity of infection (MOI), 10 MOI or 100 MOI were injected into three of five groups via intraperitoneal route. Bacteria and phages were injected at different sites. Other two groups were injected either 5×10^6 CFU of *P. aeruginosa* PAO1 only or PA1Ø only along with an additional dose of 100 μl of sterile saline to serve as control. Mice were observed at every 12 h interval up to 100 h.

In order to induce the neutropenic state of mice, 200 μg/g of cyclophosphamide (Sigma, USA) was injected i.p. at day 1, and an additional dose at day 3 into three groups of normal mice (five mice in each group) as described elsewhere (Bullwinkle and Koudelka, 2011; Satapathy and Richardson, 2011). Neutropenia was confirmed (10±3 neutrophils/cubic mm) by examining blood at day 4. One of the above groups was injected with 100 μl of *P. aeruginosa* PAO1 (5×10^3 CFU) and PA1Ø (5×10^4 PFU) i.p. through a different site. The other two groups of mice were injected the same dose of either bacteria or PA1Ø only with an additional injection of saline. Mice were observed up to 100 h.

In vivo bactericidal efficacy in different organs after PA1Ø treatment

To assess the bactericidal efficacy of PA1Ø *in vivo,* 100 μl bacterial inoculum containing 5×10^6 CFU *P. aeruginosa* PAO1 and 100 μl of PA1Ø containing 5×10^7 PFU (10 MOI) was administered i.p. to a group of 18 healthy mice, 3 mice at each time point (0.5, 12, 24, 48 and 96 h post infection) were sacrificed. Samples from blood, lungs, liver, and spleen from each mouse were collected aseptically. One milliliter of blood or 1 g of organ samples were mixed with 1 mL of PBS and homogenized using a sterile tissue homogenizer (Wheaton, USA). One hundred microliters of the diluted blood or homogenized samples from organs were serially diluted and plated on to cetrimide agar plates (MB cell, Korea), then incubated for 24 h at 37°C. The mean bacterial CFU/ml or CFU/g was calculated from triplicates. Homogenized sample filtrates were also used for plaque assay as described below, mean phage PFU calculated from three independent results, these data were used in pharmacokinetic study in presence of host bacteria. CFU counting was also done from the organ samples of 1 MOI treated dead mice for further comparison.

Pharmacokinetics of PA1Ø

Pharmacokinetic study for PA1Ø was performed by detecting phages from the blood and tissue samples in different time periods as described elsewhere (Heo *et al.*, 2009; Uchiyama *et al.*, 2009). One hundred microliters of PA1Ø (5×10^7 PFU) was given i.p to non-infected groups of mice. Three mice from each group were sacrificed at 0.5, 12, 24, 48, 72 and 96 h of post administration. Samples from blood, lungs, liver and spleen were collected aseptically then homogenized with 1 ml PBS using a sterile tissue homogenizer as described above. Then, the samples were centrifuged in 13000 rpm for 10 min at 4°C, supernatants were filtered using a 0.45 μm membrane filters, and the filtrates were serially diluted for the determination of PFU. Plaque assay was performed as described above and mean PFU from triplicate samples was determined.

Half-lives of PA1Ø in different organs and blood of normal and *P. aeruginosa*-infected mice were calculated by noncompartmental methods, using WinNonlin Pro version 5.2 (Pharsight, USA). The data from 0.5 to 96 h were used for those calculations. Mean data were analyzed by paired t-test for their statistical significance.

In vitro assessment of bactericidal activity of polymorphonuclear neutrophils (PMN)-phage co-treatment

In order to obtain PMN, 6 ml of peripheral venous blood was drawn from a healthy volunteer in a tube treated with heparin (BD Vacutainer, USA). PMN were separated by a rapid one step density gradient centrifugation technique as described elsewhere (Ferrante and Thong, 1978; Kalmar *et al.*, 1988) with a slight modification by using Histopaque 1119 and 1077 (Sigma–Aldrich, USA) reagents. Total leukocyte count was performed in a Neubauer improved counting chamber after the separation of cells by diluting 20 μl in 380 μl of Turk's fluid. Differential leukocyte count was performed by examining Giemsa stained smears under the microscope. One hundred microliters of *P. aeruginosa* PAO1 (5×10^6 CFU/ml), 100 μl of PA1Ø (5×10^7 PFU/ml), and 200 μl of PMN (PA1Ø + PMN) (7.2×10^5 cells/ml) were added in 2 ml RPMI 1640 medium (Thermo scientific, USA) with 10% normal autologous serum (Repine *et al.*, 1980) into three sterile 15-ml size polystyrene tubes (SPL Life Science, Korea) separately. Similarly, in another 3 tubes, the same dose (as above) of only phage and bacteria without PMN (only PA1Ø) were added in 2 ml RPMI medium with 10% fetal calf serum. Tubes were incubated at 37°C by continuous shaking at 120 rpm. One hundred microliters of the samples were seeded on cetrimide agar plates every hour up to 7 h. Plates were incubated at 37°C. CFU counting was done after 24 h of incubation. This experiment was undertaken for three consecutive days independently. Fresh blood samples were collected

each day from the same donor. Each day's mean CFU counting was calculated from triplicate results. Final mean CFU was calculated from three days' independent results. Paired t-test was done to determine the statistical significance.

Results

Protective efficacy of PA1Ø in immune-competent and neutropenic mice infected with *P. aeruginosa* PAO1 strain

From mortality assay, 5×10^6 CFU/mice was the minimum lethal dose of *P. aeruginosa* PAO1 in immune-competent mice with 80% mortality, although the 20% surviving mice also faced serious consequences of septicemia before final recovery. Similarly 5×10^3 CFU/mouse was the minimum lethal dose (with 100% mortality) in cyclophosphamide treated neutropenic mice.

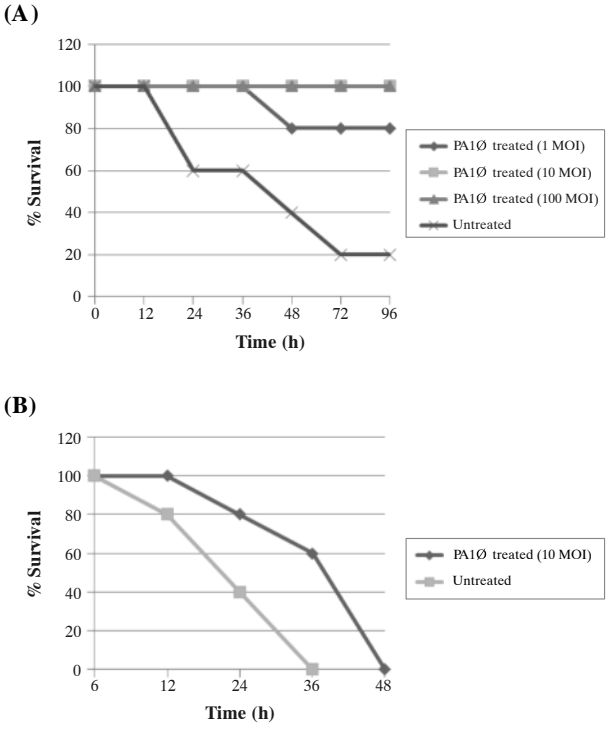

Fig. 1. Protective effects of PA1Ø in normal (A) and neutropenic (B) mice. Normal mice were injected with 5×10^6 CFU *P. aeruginosa* PAO1 and treated with either 5×10^6 PFU (1 MOI), 5×10^7 PFU (10 MOI) or 5×10^8 PFU (100 MOI) PA1Ø right after bacterial infection from different sites. A total of 75 mice were used, twenty five mice in each independent experiment (5 mice in each group as described in Materials and Methods). Neutropenic mice were injected with 5×10^3 CFU *P. aeruginosa* PAO1 and treated with 5×10^4 PFU (10 MOI) PA1Ø. A total of 45 mice were used, fifteen mice in each independent experiment (5 mice in each group as described in material and method) , three independent experiments showed the consistent results. All of the mice receiving either 5×10^7 PFU (10 MOI) or 5×10^8 PFU (100 MOI) of PA1Ø were successfully protected, mice treated just with 1 MOI of PA1Ø were also 80% protected. All phage treated neutropenic mice infected with bacteria died within 48 h post infection, 60% of phage treated mice survived 12 h longer than untreated mice.

Protective efficacy of PA1Ø in immune-competent and neutropenic mice was evaluated (Fig. 1). All of the mice receiving either 5×10^7 PFU (10 MOI) or 5×10^8 PFU (100 MOI) of PA1Ø were successfully protected from the *P. aeruginosa* infection without any signs and symptoms of illness. Eight of ten immune-competent mice which were treated with 1 MOI of PA1Ø survived until 96 h of post infection (Fig. 1A).

However, PA1Ø treatment could not protect the neutropenic mice but extend their lives for additional 12 h (Fig. 1B). All phage treated neutropenic mice infected with bacteria died within 48 h post infection. These mice developed severe clinical conditions of septicemia after 12 h followed by a moribund state and finally death. There were no remarkable differences in morbidity and mortality between the phage treated and untreated control groups, the only difference noted was that, 60% of phage treated mice survived 12 h longer than untreated mice. Neutropenic mice injected with phages only did not produce any adverse effect.

The mean CFU in blood and organ samples at different time points is shown in Fig. 2 that single dose of PA1Ø (10 MOI) substantially reduced the viable bacteria in blood and organs of normal mice in 48 h. None of the sample contained any viable bacteria at 48 h and thereafter. Mean CFU of dead mice from 1 MOI treated group showed much higher viable bacteria than in the samples from surviving mice (2×10^8, 2.5×10^9, and 5×10^7 in lungs, liver and spleen, respectively) – confirming bacterial infection as cause of death.

Pharmacokinetics of PA1Ø in the presence or absence of host bacteria

In the absence of host bacteria, the decreasing patterns of phages showed downhill type without stationary phase from initial injection time to 72 h in blood, lungs, and liver (Fig.

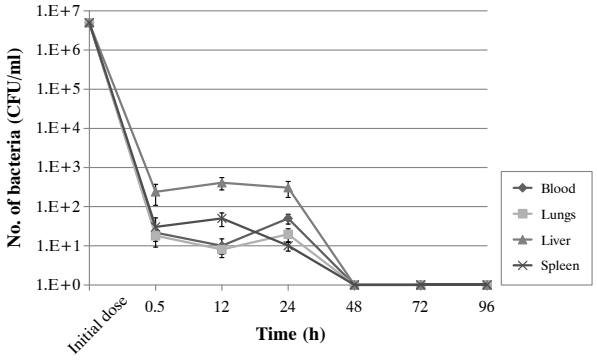

Fig. 2. Bactericidal effect of PA1Ø in samples from phage-treated normal mice. Bacterial inoculum containing 5×10^6 CFU *P. aeruginosa* PAO1 and 100 µl of PA1Ø containing 5×10^7 PFU (10 MOI) was administered i.p from different sites. Mice were sacrificed at different time points. One hundred microliters of the diluted blood or a homogenized sample from each organ was serially diluted, plated on to a cetrimide agar plates, and incubated for 24 h at 37°C. None of the sample contained any viable bacteria at 48 h and thereafter. A total of 54 mice were used (9 mice at each time point). The data represent the mean value from three independent experiments. At the same time homogenized organ sample filtrates were serially diluted and plaque assay was performed as described in Materials & Methods, these data are shown in Fig. 3A.

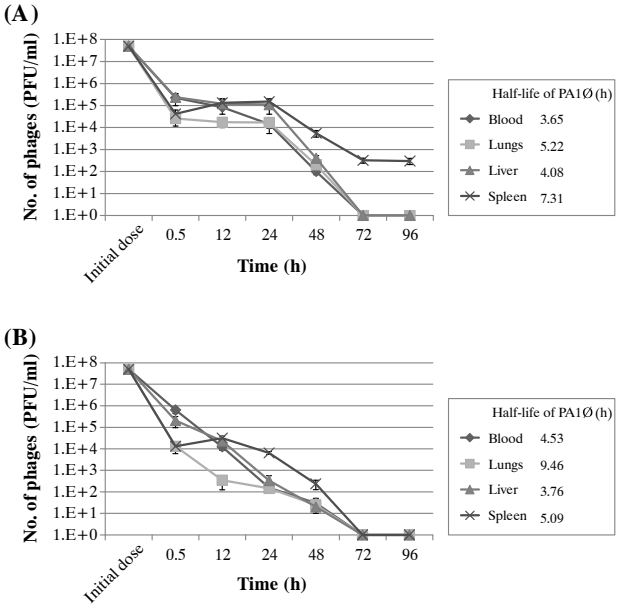

Fig. 3. PA1Ø pharmacokinetics in presence (A) or absence (B) of host bacteria in normal mice. Mice were sacrificed at 0.5, 12, 24, 48, 72 and 96 h post administration. Homogenized organ sample filtrates were serially diluted and plaque assay was performed as described in Materials and Methods. A total of 54 mice were used. The results represent the mean value from three independent experiments. The mean PFU values obtained from all the organs and blood in presence of host bacteria (A) were higher at 12 h (P-value < 0.01) and 24 h (P-value < 0.05) than in absence of host bacteria (B).

3B). However, the number of phage plaques was increased from 0.5 h to 12 h in spleen and decreased again after 12 h post injection. Phages were not found in all samples after 72 h. Half-lives of PA1Ø were 4.53, 9.46, 3.76, and 5.09 h in blood, lungs, liver, and spleen, respectively. Contrary to the pharmacokinetics of PA1Ø in the absence of host bacteria, PFU of PA1Ø in the infected mice were decreased approx. 1000 fold from the initial inoculum PFU at 30 min post injection, but the level of phage concentration continued from 30 min to 24 h post injection in blood and three organs (Fig. 3A). In the duration between 24 and 48 h, numbers of PA1Ø were decreased slightly in three organs and blood, but those were maintained over 10^2 PFU per 1 ml of blood or 1 g of the three organs. After 48 h of the injection, the number of the phages in blood, lungs, and liver was significantly decreased and the phage plaque was not found at and after 72 h. However, the number of phages in spleen was lasted until 96 h with small loss. The decreasing patterns of the number of PA1Ø in presence of host bacteria was very similar with those of the number of host bacteria; decreasing in 30 min, lasting between 0.5-24 h, and decreasing after 24 h. As shown in (Fig. 2), viable host bacteria were not found after 48 h. After the time point of complete loss of host bacteria, phages were significantly decreased. The phage PFU results obtained from all the organs and blood in presence of host bacteria (Fig. A) were found significantly higher at 12 h (P-value < 0.01) and 24 h (P-value < 0.05) than in absence of host bacteria (Fig. B). Results at 0.5 h and after 48 h were statistically insignificant. Half-lives of PA1Ø were 3.65, 5.22, 4.08, and 7.31 h in blood, lungs, liver, and spleen, respectively.

In vitro assessment of bactericidal activity of PMN-phage co-treatment

Despite showing 100% protective effect in the normal mice, PA1Ø could not protect any neutropenic mouse from infection. We assumed that this result might be due to the emerging of phage-resistant mutants and lack of sufficient neutrophil phagocytic cells. To support this hypothesis, the synergistic role of neutrophils with PA1Ø was tested *in vitro.* Neutrophils were separated from human blood as described in Materials and Methods, total leukocyte count was found to be 8000± 1000 cells/cubic mm and differential leukocyte count showed 90±5% PMN. As shown in (Fig. 4), the mean CFU value was significantly lower in PA1Ø+PMN treated samples than only PA1Ø treated samples during the entire period of 1 to 7 h (P-value ≤ 0.02). The difference in viable bacterial number was only 6 fold higher in only PA1Ø treated samples after 1 h but it reached more than 226 fold at 6 h. The lowest number of viable bacteria in only PA1Ø treated samples was observed at 3 h whereas it was observed in PA1Ø+PMN treated samples at 5 h. Phage-resistant mutants number increased after 4 h in only PA1Ø treated samples but it took place after 6 h in PA1Ø+PMN treated samples. The extent of population doubling of phage-resistant mutants was much more rapid in only PA1Ø treated samples than in PA1Ø+ PMN treated samples.

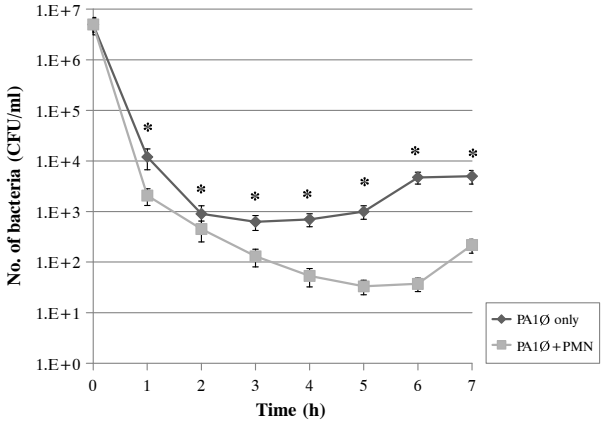

Fig. 4. Phage-neutrophil co-work for bactericidal effect *in vitro*. One hundred microliters of *P. aeruginosa* PAO1 (5×10^6 CFU/ml), 100 µl of PA1Ø (5×10^7 PFU/ml) were added in 2 ml RPMI 1640 medium either with 200 µl of PMN containing 7.2×10^5 cells/ml (PA1Ø + PMN) or without PMN (PA1Ø only) and incubated at 37°C. Samples were obtained every hour and seeded on cetrimide agar plates up to 7 h. CFU counting was done after 24 h of incubation. These results represent the mean value from three independent experiments. * The mean CFU values were significantly lower in PA1Ø+PMN treated samples than in only PA1Ø treated samples during the entire period of 1 to 7 h (P-value ≤ 0.02).

Discussion

In this study, we focused on the evaluation of *in vivo* antimicrobial efficacy of a novel *Pseudomonas* phage (PA1Ø) against *P. aeruginosa* strain PAO1 in immune-competent and neutropenic mice. We found that treatment of single dose of mono-phage (PA1Ø) showed a strong protective effect in mouse model infected with the lethal dose of *P. aeruginosa* PAO1. Phage treated mice did not produce any symptoms and signs of septicemia whereas phage untreated mice developed mild lethargy at 6 h followed by lack of physical activities with ruffled fur, hunch back posture, moribund state and finally death due to septicemia. Our results were similar to previous reports that mono-phage treatment successfully protected mice from *Pseudomonas* infection (Watanabe *et al.*, 2007; Heo *et al.*, 2009). Relevance of the protective efficacy of PA1Ø was supported by its bactericidal effect *in vivo*. Outcomes found almost consistent with some previous results where phages were utilized successfully in *in vivo* experimental models (Wang *et al.*, 2006; McVay *et al.*, 2007; Watanabe *et al.*, 2007; Heo *et al.*, 2009) and supported the use of PA1Ø against *P. aeruginosa* infection.

We also evaluated the efficacy of PA1Ø in neutropenic mice. Bacteria and phage were given i.p. as previously described; but it did not produce any protective effect in neutropenic mice. Other than a delay in mortality of 12 h, no discrepancy was noted in mortality and other clinical consequences in phage treated and untreated neutropenic mice. Since the partially purified phage lysate seemed to be totally harmless in our study in healthy as well as neutropenic mice. We suspected the failure to protect neutropenic mice was probably due to lack of PMN which can kill the remaining bacteria (even phage-resistant bacteria) after phage attack (Levin and Bull, 2004). Previously we noted the emergence of phage-resistant mutants against PA1Ø when we performed *in vitro* experiments (data not shown). One of the concerns of phage therapy is probable evolution of phage-resistant mutants by targeted bacteria (Watanabe *et al.*, 2007) but these may be cleared by host defense system *in vivo* (Levin and Bull, 2004). PMN provide first line innate defense in acute bacterial infection (Repine *et al.*, 1980) – they probably phagocytose and kill evolving phage-resistant mutants in immunocompetent hosts before phage-resistant mutants can multiply sufficiently. We evaluated the role of neutrophils in phagocytosing bacteria by simultaneous interaction of phage and neutrophils against *P. aeruginosa* using *in vitro* model. Lower viable bacteria were detected in phage and neutrophils co-treated (PA1Ø+PMN) samples than in only phage (PA1Ø) treated samples as incubation time increased. Killing effect in PA1Ø+PMN seemed aggressive continuously up to 6 h. The slight increase of viable bacteria after 6 h could be happened caused by decreasing phagocytic killing to overgrowing mutant population as a result of PMN apoptosis. It has been reported that pyocyanin of *Pseudomonas* prompts neutrophil apoptosis (Usher *et al.*, 2002). The results suggested that the continuous turnover of PMN in *in vivo* system support the successful phage therapy and the eradication of phage-resistant mutants. There is little evidence regarding the interaction between phages and mammalian cells – nonetheless, the bactericidal action of phages promotes the turnover of neutrophils by inducing proinflammatory cytokines and lactoferrin release; which further induces colony stimulating factors to recruit neutrophil precursors from bone marrow (Weber-Dabrowska *et al.*, 2002). This explanation highlighted the phage-neutrophil synergistic bactericidal role in *in vivo* system. To our knowledge, this is the first report regarding the phage-phagocyte co-work with coinciding interaction against *P. aeruginosa* in *in vitro* model. Indeed, our findings will be helpful for further understanding of the role of PMN in phage therapy endeavor. Nevertheless, phage therapy had been suggested to be effective in immune-compromised hosts against *P. aeruginosa* bacteremia (Borysowski and Gorski, 2008). Our results suggest the high possibility of emerging phage-resistant mutants by using only mono – phage in neutropenic subject even though it possesses a strong protective effect in immune-competent hosts. Interestingly, our results followed the theoretical model projected elsewhere (Levin and Bull, 2004). It has been suggested that, phage resistance can be prevented by using phage cocktails (Fu *et al.*, 2010) – likewise phage-antibiotic synergy had been observed *in vitro* (Comeau *et al.*, 2007). The therapeutic efficacy of phage cocktail in neutropenic host models needs to be further investigated in this regard.

We carried a parallel phage pharmacokinetics study in the presence or absence of target bacteria *in vivo*. Our results showed differences of phage clearance kinetics in all samples between bacterial infected and non-infected groups (Fig. 3A, B). The number of phages in non-infected host speedily decreased subsequently from 12 h and this more rapid decline of phages in non-infected group than in infected group evidenced their capability of *in vivo* propagation when the target host is available. The results were similar to a related previous study (Wang *et al.*, 2006). Since the bacterial host was eliminated after 24 h and the phages after 48 h, it seemed a quite relevant to this kinetics. Interestingly, phages were detected at 72 and 96 h in spleen samples from the infected group, which may be due to the sequestration of the phages in the spleen. Kinetics of *Pseudomonas* phages varied with the results of Mcvay (McVay *et al.*, 2007) due to its rapid clearance rate but it seemed consistent with the results of Heo (Heo *et al.*, 2009). Comparison in half-lives of PA1Ø showed little difference between infected and non-infected mice model and this might be due to complete clearance of host bacteria at and after 48 h in infected mice. If the host bacteria were maintained consistently until 72 or 96 h in infected mice, the half-life of PA1Ø in infected mice could thereby be increased.

In conclusion, PA1Ø can be possibly utilized in future phage therapy endeavors since it exhibited strong protective effects against virulent *P. aeruginosa* infection. This study also suggested that single phage therapy to neutropenic patients or animals can prolong life to some extent but may not be a promising choice for the control of the bacterial pathogens.

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