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Investigation of PhiKZ phage therapy against *Pseudomonas aeruginosa* in mouse pneumonia model

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Background/aim: The aim of this study was to investigate the effects of PhiKZ phage therapy and meropenem alone or combined treatments in a pneumonia mouse model induced by the *Pseudomonas aeruginosa* PAO1 strain. The cross-talk between lungs and kidneys was also determined.

Materials and methods: The systemic, lung-specific, and kidney-specific inflammation levels and the bacterial load in lung tissue and biochemical parameters were investigated after PhiKZ phage therapy and meropenem alone or combined treatments in a pneumonia mouse model induced by the *P. aeruginosa* PAO1 strain. The cross-talk between lungs and kidneys was also determined by measuring plasma levels of glycocalyx components.

Results: The greatest reduction in lung bacterial load was obtained with the combined use of the PhiKZ phage and meropenem. The C-reactive protein level in the patient group was significantly higher than in the treatment groups and decreased after treatment. Serum interleukin IL-6 levels were statistically significantly higher than in the phage serum and phage + meropenem groups. Pulmonary infection can trigger proinflammatory cytokines such as IL-6, TNF-α, and IL-1β. Increased cytokines trigger insulin resistance in the liver. Lung infection triggers liver inflammation because there is communication between the lungs and liver.

Conclusion: Elevated proinflammatory cytokines due to infection were decreased because of the reduced burden of bacterial load after treatment. This study might have proved communication between lungs and kidneys related to proinflammatory cytokines.

Key Words: Phage therapy, bacterial load, *Pseudomonas aeruginosa* PAO1, PhiKZ phage, pneumonia, mice model

1. Introduction

Phage therapy is the therapeutic use of bacteriophages to eliminate or weaken pathogenic bacteria. Phage therapy has been given reconsideration due to the inadequacy of antibiotic treatment in the increased numbers of multidrug-resistant infections (1). The therapeutic use of phages in the treatment of bacterial infections began in humans immediately after their discovery (2,3–6). The first phage therapy study was conducted by d'Herelle in 1919 (7). Despite the declining phage treatment practices in the world after the discovery of antibiotics, phage treatment practices continued at the Eliava Institute in Georgia and the Hirszfeld Institute in Poland (8).

Pseudomonas aeruginosa is a gram-negative, motile, nonfermentative, aerobic and anaerobic bacterium that can populate a variety of environments such as soil, water, plants, and animals (9). *P. aeruginosa* is an opportunistic human pathogen that causes chronic and acute infections of burn wounds and respiratory and urinary tracts with significant morbidity and mortality rates (10). In addition, the major cause of morbidity and mortality in individuals with cystic fibrosis is *P. aeruginosa*; it is one of the main causes of hospital-acquired infections and lung infections. Moreover, multidrug-resistant rates are particularly high in *P. aeruginosa*, which severely limits the therapeutic options available to treat infected patients (11). The inadequacy of antibiotics has led physicians to search for new treatment strategies, one of which is phage therapy. The PhiKZ phage is a giant bacteriophage in the family *Myoviridae* with a circular genome that infects *P. aeruginosa* (12). Phages such as PhiKZ are indispensable components of commercial therapeutic phage mixtures because of their plentiful growth (13).

Abedon and Thomas-Abedon (2), Abedon et al. (14), and Abedon (15) revised the use of phage therapy in lung disease. Morrison and Gardner conducted the first study

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in 1936 (16). They successfully applied local phage therapy in *Bacillus*-induced lung disease. The topical treatment of staphylococcal phage therapy in newborns and children was investigated in 1938 (17). Sakandelidze and Meipari treated bronchitis and lung abscesses with subcutaneous or topical applications of phages (18). Phage therapy uses a combination of antibiotics and Sulakvelidze et al. (19) performed polyvalent phage therapy.

P. aeruginosa has been used in phage therapy trials because it has multidrug resistance and causes a biofilm. In a study by Wright et al. (20), *P. aeruginosa* was topically administered (6×10^5 pfu/mL) to patients with chronic otitis and was reported to reduce bacterial load. The successful treatment of infections caused by *P. aeruginosa* and *Staphylococcus aureus* in patient with burns was done by using phage cocktails (21,22). Yilmaz et al. (23) used methicillin-resistant *Staphylococcus aureus* and *P. aeruginosa* in rats using an implant-related infection model. Sb-1 and PAT phages were applied in phage treatment. The protective usage of ϕMR11 bacteriophages against *Staphylococcus aureus* infection in mice was successfully conducted (24). Phages and combined use of antibiotics were observed to prevent the formation of biofilms. Combined usage of phages and antibiotics effectively reduced the number of bacteria in in vitro and in vivo experiments (25,26).

Sulakvelidze et al. (19) reviewed phage therapy in lung diseases. Phages are usually administered topically, systemically, or orally in the treatment of pulmonary infections. Topical phage therapy and systemic (parenteral) administration may be administered individually or together, and in some cases by direct injection in a rat model of pulmonary infection (27,28).

The aim of this study was to test the effects of PhiKZ and meropenem alone or combined treatments in a pneumonia mouse model induced by the *P. aeruginosa* PAO1 strain. For an integrative investigation of the effects of phage therapy, we measured routine biochemistry parameters in addition to determining bacterial load in the lungs. Furthermore, glycocalyx degradation was assessed by measuring plasma levels of hyaluronan (glycocalyx compartment), and plasma levels of tumor necrosis factor (TNF)-α and interleukin (IL)-6 were measured as markers of inflammation. Moreover, the cross-talk between the lungs and kidneys was evaluated by measuring cytokines in kidney tissues.

2. Materials and methods

2.1. Ethical statements and animals

All animal studies were performed in accordance with the Regulation on Working Procedures and Principles of Ethical Committees of Animal Experiments (Ministry of Forestry and Water Management, Turkey, 5199, 2004). The study was approved by the İstanbul University Animal Experiments Local Ethics Committee (İÜ HADYEK, 2014/67). Thirty male C57/BL6 mice weighing 21–23 g were used in this study. The animals were obtained from İstanbul University's Institute of Experimental Medicine (DETAE). Mice were housed under standard laboratory conditions at DETAE.

2.2. Bacterial and bacteriophage strains and culture conditions

P. aeruginosa PAO1 (ATCC 15692) and PhiKZ (ATCC BAA28 B2) phages were purchased from the American Type Culture Collection (ATCC) and animated and stock cultures were prepared according to the protocol of the ATCC. All isolates were stored at –20 °C with 15% glycerol.

2.3. PFU calculation

Phage titer was checked using the double agar overlay technique of Adams (29) and expressed as plaque forming units/mL, as previously described by Adams. The *P. aeruginosa* PAO1 strain was propagated from a stock solution. One to two drops of *P. aeruginosa* PAO1 were add to 2.5 mL of 0.5% molten agar and mixed and spread in tryptic soy agar petri dishes. After hardening the overlay, serial dilutions of 1 mL of phage prepared with 0.9 mL of TSB and 0.1 mL of phage were poured onto the surface of each plate. The plate was tilted to ensure the entire surface was covered. After the surface dried, the petri dishes were incubated at 37 °C for 24 h. Plaque-forming units (pfu) of phage were calculated using the following formula:

pfu/mL = number of plaques / d × V.

v = volume of phage added to petri dishes, d = dilution.

2.4. Experimental groups

The mice were divided into 5 groups of 6 individuals. Experimental groups and their applications are summarized in the Table. In the pneumonia group, mice were inoculated intranasally with 10 µL of 5×10^8 cfu/mL *P. aeruginosa* PAO1 strain in order to induce a pneumonia model as previously described in detail by Campodónico et al. (30). In the control group, mice were inoculated with phosphate-buffered saline (PBS). In the pneumonia + PhiKZ group, mice were inoculated with *P. aeruginosa* PAO1 (5×10^8 colony-forming units (cfu)/mL) and phiKZ phage (1.5×10^8 pfu/mL). In the pneumonia + meropenem group, mice were inoculated with *P. aeruginosa* PAO1 (5×10^8 cfu/mL) and meropenem (100 mg/kg) intraperitoneally. In the pneumonia + PhiKZ + meropenem group, mice were inoculated with *P. aeruginosa* PAO1 (5×10^8 cfu/mL) and meropenem (100 mg/kg intraperitoneal) and phiKZ phage (1.5×10^8 pfu/mL).

2.5. Microbiologic and biochemical measurements

Mice were anesthetized using an intraperitoneal injection of 65 mg/kg ketamine and 13 mg/kg xylazine 24 h after inoculation. First, bronchoalveolar lavage (BAL) fluid was

Table. Experimental groups and treatments.

Groups	Inoculation	Treatment 1 (2nd hour)	Treatment 2 (12th hour)
1- Control (n = 6)	10 µL of sterile PBS	PBS	PBS
2- Pneumonia (n = 6)	*P. aeruginosa* PAO1 (5×10^8 cfu/ml)	PBS	PBS
3- Pneumonia + PhiKZ (n = 6)	*P. aeruginosa* PAO1 (5×10^8 cfu/mL)	PhiKZ phage (1.5×10^8 pfu/mL)	PhiKZ phage (1.5×10^8 pfu/mL)
4- Pneumonia + meropenem (n = 6)	*P. aeruginosa* PAO1 (5×10^8 cfu/mL)	Meropenem (100 mg/kg intraperitoneal)	Meropenem (100 mg/kg intraperitoneal)
5- Pneumonia + PhiKZ + meropenem (n = 6)	*P. aeruginosa* PAO1 (5×10^8 cfu/mL)	PhiKZ phage (1.5×10^8 pfu/mL) + meropenem (100 mg/kg intraperitoneal)	PhiKZ phage (1.5×10^8 pfu/mL) + meropenem (100 mg/kg intraperitoneal)

taken under anesthesia. A left main bronchial catheter was placed and washed with 1 mL of isotonic NaCl solution. In each wash, the solution was rapidly injected and collected slowly. After the bronchoalveolar lavage fluid was taken, blood was taken from the heart. Entering the heart with a 1-mL syringe and with all the blood taken in one shot, the animals were sacrificed and the kidney and lung tissues were taken for histopathological and biochemical examination. Lungs and kidneys were harvested and blood samples was centrifuged at 5000 rpm for 7 min at 4 °C. After removal of erythrocytes, plasma samples were stored with kidney samples at –80 °C until measurement day. Lung samples were immediately used for bacterial load measurement.

2.6. Determination of bacterial load

Homogenized lung tissues were centrifuged at 1400× *g* for 10 min. The supernatant was collected and serially diluted to 10^6. One hundred microliters of diluted samples were plated onto Muller-Hinton agar. Bacteria colonies were counted after 24 h of incubation at 37 °C. Colony-forming units (cfu) were determined using the formula:

$$Cs = \frac{Z}{Vtot} \times Vs$$

Cs: Estimated number of cfu.

Z: Total number of colonies.

Vs: Selected reference volume (1 mL or 100 mL).

Vtot: Collected amount of colonies from dilution counted from petri dishes.

2.7. Biochemical measurements

C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, glucose, lactate, and creatinine levels were determined as routine biochemical measurements. Plasma levels of IL-6, IL-1β, and TNF-α were measured using an enzyme-linked immunosorbent assay (ELISA) as markers of systemic and tissue inflammation (e-BioScience, USA) following the directions of the kit's manufacturer and concentrations were measured at 405 nm as absorbance. Hyaluronan is the main component of endothelial glycocalyx, in the extracellular matrix, and alterations in its concentration in plasma can be attributed to glycocalyx volume loss. Hyaluronan concentrations were determined using the Sun Red hyaluronic acid test kit (Sun Red, China), which is based on an enzyme-linked hyaluronic acid binding protein assay. The kit's procedure was applied and the absorbance values were measured at 405 nm. Lung homogenate protein content was quantified using Bradford's assay (Bio-Rad, USA).

2.8. Statistics

Datasets are presented as means ± SEM and were analyzed using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). Groups were compared using one-way analysis of variance and, when appropriate, post hoc analyses with Tukey tests. Statistical significance was considered as $P < 0.05$.

3. Results

3.1. Bacterial load

Bacterial load was measured from homogenized lung tissues through the recovery of PAO1 (Figure 1A). The bacterial load of the pneumonia group was 5.82 ± 0.07 log *P. aeruginosa* PAO1. The bacterial load of the group treated with PhiKZ was 4.4 ± 0.14 log. The bacterial load of the meropenem and meropenem + PhiKZ treatment groups were 4.84 ± 0.08 log and 4.33 ± 0.03 log, respectively. Bacterial load was reduced by about 89% in the PhiKZ treatment group. Bacterial load reduction of the meropenem and meropenem + PhiKZ treatment groups was 96% and 97%, respectively. Statistically significant bacterial load reduction ($P < 0.05$) was observed in the treatment groups (Figure 1A).

3.2. Routine biochemical results

Measurements of CRP, ALT, AST, urea, glucose, lactate, and creatinine levels were performed (Figures 1B–1D).

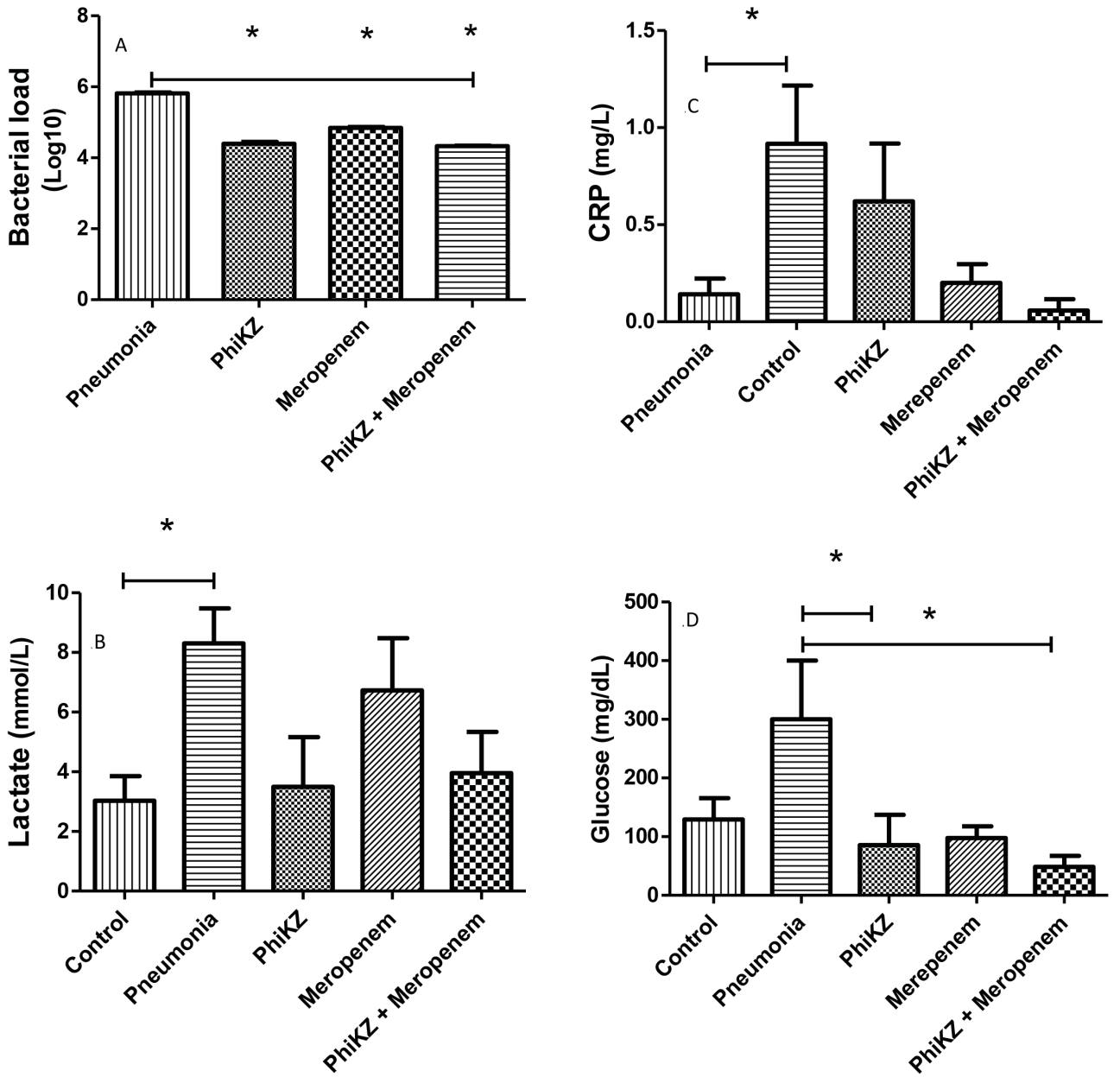

Figure 1. Bacterial load and biochemical measurements: A) bacterial load in lung homogenate, B) lactate, C) CRP, D) glucose.

CRP level (Figure 1C) was found statistically significant at 0.92 ± 0.3 mg/L in the pneumonia group ($P < 0.05$ vs. control). CRP levels of the phage, meropenem, and phage + meropenem groups were lower than in the pneumonia group. The glucose level of the pneumonia group (Figure 1D) was determined as 300.4 ± 89.5 mg/L and a statistically significant difference was found between the pneumonia and treatment groups ($P < 0.05$). The lactate measurements (Figure 1B) showed the highest mean value in the pneumonia group (8.3 ± 1.2 mmol/L) and the lowest mean value in the control group (3.03 ± 0.8) ($P < 0.05$, pneumonia vs. control group). When ALT, AST, urea, and creatinine levels were investigated in serum samples, no statistically significant difference was found between pneumonia and the other treatment groups.

3.3. Inflammatory and glycocalyx degradation products

ELISA results are presented in Figures 2A–2D. There was a significant difference between IL-6 values (Figure 2D) in the control and pneumonia groups ($P < 0.05$). In addition, posttreatment IL-6 levels showed a statistically significant decrease ($P < 0.01$). In the phage treatment group, the level of IL-6 was measured as 306.8 ± 67.1 pg/mL and

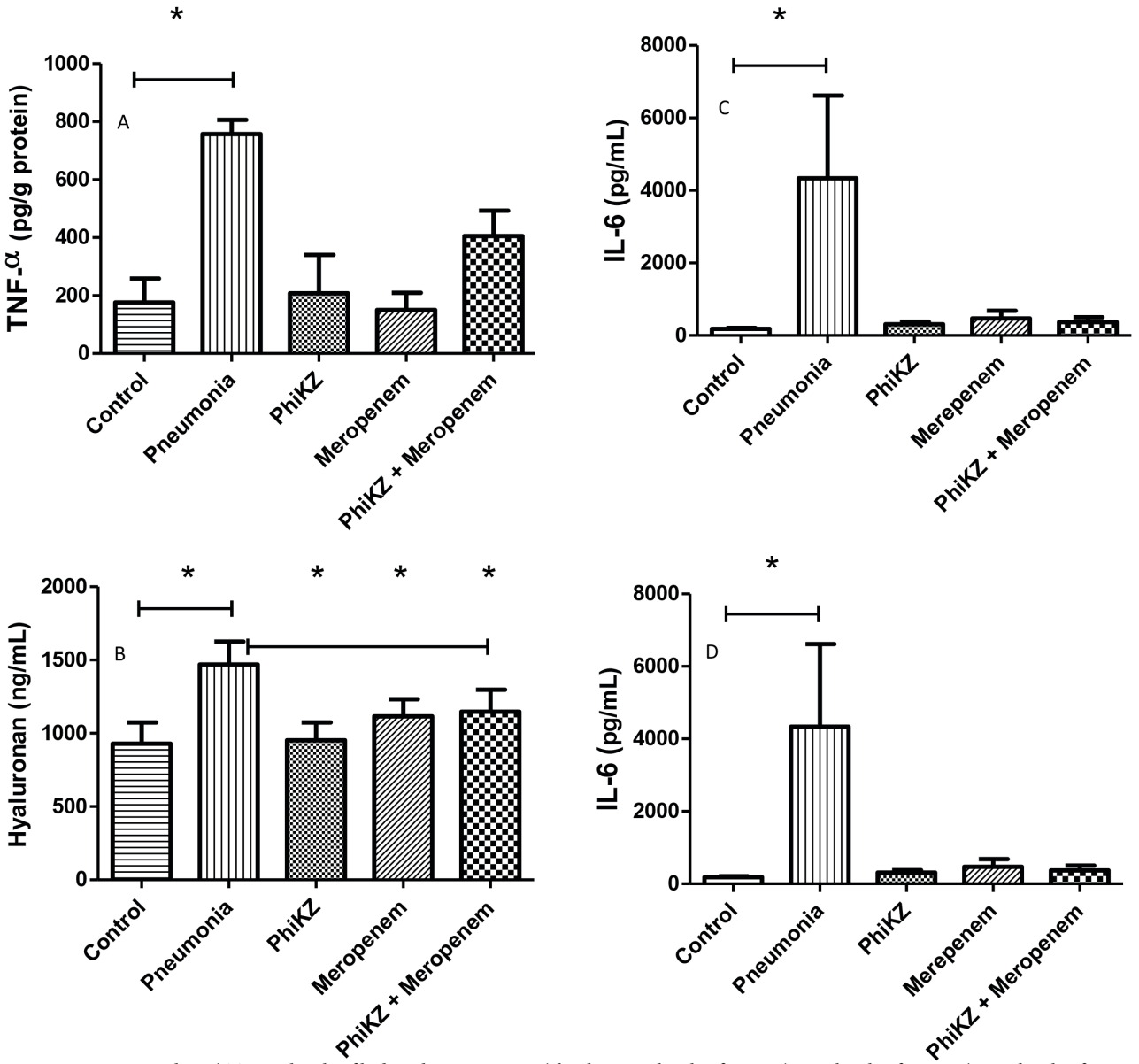

Figure 2. ELISA results: A) TNF-α levels of kidney homogenate, B) hyaluronan levels of sera, C) IL-6 levels of BAL, D) IL-6 levels of sera.

was found to be lowest compared to other treatment groups. Serum IL-1β levels were measured highest in the pneumonia group (24.38 ± 15.6 pg/mL) and lowest (8.578 ± 1.3 pg/mL) in the phage + meropenem group. IL-1β levels were decreased in all treatment groups, but no statistically significant difference was found. The highest TNF-α level was measured in the pneumonia group as 13,630 ± 9509 pg/mL. In the treatment groups, the lowest value obtained was 1245 ± 1102 pg/mL in the phage + meropenem treatment group. A decrease in TNF-α levels (Figure 2A) was observed in the treatment groups. Hyaluronan measurements (Figure 2B) in serum samples were 1469 ± 157 ng/mL in samples from the pneumonia group. In the phage-treated group, it was 952 ± 123.0 ng/mL, which was the lowest among the groups. A statistically significant decrease was observed between the control and pneumonia groups ($P < 0.05$).

3.4. Measures related to the inflammatory process and glycocalyx in BAL

In IL-6 measurements (Figure 2C) performed on BAL samples, the highest value was measured as 26,485 ± 19,242 pg/mL in samples from the pneumonia group. The mean IL-6 level was measured as 6574 pg/mL in the treatment groups. The value in the pneumonia group was statistically higher than in the control group ($P < 0.05$, t-test). Levels of IL-1β were 1319 ± 769 pg/mL in the pneumonia group and

130.7 ± 68 pg/mL in the control group. The levels of IL-1β in the control and treatment groups were significantly lower than in the pneumonia group; however, this was not statistically significant.

The highest value (1261 pg/mL) was found in samples from the pneumonia group in the TNF-α assay in BAL samples. The mean TNF-α value in the treatment groups was 340 pg/mL. There was a decrease in the TNF-α value in the treatment groups compared with the pneumonia group, but no statistically significant difference was found.

3.5. Measures Related to the inflammatory process and glycocalyx in the kidneys

The average IL-6 level in samples from the pneumonia group was 4115 ± 882 pg/mL. In the control samples, it was 1163 ± 1115 pg/mL. The mean value in the treatment groups was found as 2268 pg/mL. There was a significant difference between the pneumonia group and the group treated with meropenem ($P < 0.05$). IL-1β was found as 1113 ± 363 pg/mL in samples from the pneumonia group and 255 pg/mL in the treatment groups. There was no statistically significant difference between the groups. The mean TNF-α level in samples from the pneumonia group was 756.8 ± 49.6 pg/mL. In the treatment groups, there was a decrease (254 pg/mL) according to the pneumonia group. There was a statistically significant difference between the control group and the patient group ($P < 0.05$). The mean hyaluronan value of the pneumonia group was 152.7±16.5 ng/mL. The mean value in the treatment groups was 132.7 ng/mL.

4. Discussion

P. aeruginosa is one of the main causes of hospital-acquired infections with a high rate of morbidity and mortality. It is also an opportunistic pathogen that can lead to bacterial infection in patients who are immunocompromised (cancer, HIV, cystic fibrosis, burn infections). Treatments are difficult because of natural antibiotic resistance mechanisms. Experimental studies have been carried out in recent years on the use of phages in the treatment of lung infections caused by *P. aeruginosa* (31).

Bacterial load reduction was about 89% in the PhiKZ treatment group, and 96% ($P < 0.05$) and 97% ($P < 0.05$) bacterial load reductions were observed in the meropenem and meropenem + PhiKZ treatment groups, respectively. A significant reduction in bacterial load was obtained in the lungs in the treatment groups. A statistically significant difference ($P < 0.05$) was observed between the pneumonia group and other groups. Combined treatment of phage + meropenem was found as the most effective treatment against *P. aeruginosa* pneumonia in this mouse model.

In this study, serum CRP, lactate, and hyaluronan levels between the pneumonia and control groups were statistically significant. A significant reduction in bacterial load was obtained in the lungs in the treatment groups. The IL-6 level ($P < 0.05$, t-test) in BAL samples showed a statistically significant difference between the pneumonia group and control group. There were statistically significant differences in renal homogenates between the pneumonia and control groups in levels of IL-6 ($P < 0.05$) and TNF-α ($P < 0.05$).

Alemayehu et al. (32) used φMR299-2 and φNH-4 phages to eliminate *P. aeruginosa* in the mouse lung and cystic fibrosis lung cells. They found a 3–4 log decrease in bacterial load within 6 h. In our study, a decrease of 1.5 log was observed. Pires et al. (31) used phages in biofilm treatment and observed a significant decline after 6 h. Torres-Barceló et al. (33) used a phage and antibiotic combination against *P. aeruginosa*, similar to this study. They reported that combined therapy was more effective than the phage alone or antibiotics alone. Their result is consistent with the results of the present study. Phage therapy and antibiotics affect the bacterium through different mechanisms; therefore, combined treatment reduces resistance formation and can be used against pathogens with high resistance (34,33). A better understanding of the pharmacodynamics of combined therapy will allow the identification of new treatment strategies in multiresistant bacterial infections. The YH6 phage was used as an alternative phage therapy agent against multidrug-resistant *P. aeruginosa* in a murine haemorrhagic pneumonia model (35).

PAK-P and P3-CHA phages were also tested in a multidrug-resistant *P. aeruginosa* mouse pneumonia model for treatment, and decreases of mortality rate and bacterial load after 18 h of treatment were observed (36). Debarbieux et al. (37) observed a decrease in mortality and lung inflammation as a result of treatment with the PAK-P1 phage in a *P. aeruginosa* mouse pneumonia model. Combined treatment with LUZ7 phage and streptomycin was administered against nosocomial pathogen *P. aeruginosa*. Coadministration of the phage and antibiotics led to a stronger reduction in bacterial count than the phage or antibiotic treatment alone; moreover, unlike single therapies, after combined treatment, the bacteria did not reach their old levels.

In our study, the greatest decrease in bacterial count was observed in the combined administration of the phage and meropenem (97% decrease, $P < 0.05$). Similarly, the use of antibiotics caused a decrease in the number of bacteria, and the SBWu2 phage alone reduced the number of bacteria. However, the combined use of the phage and antibiotics caused the greatest decrease (38). It was also reported that bacterial resistance formation was less with the combined treatment (38). Verma et al. (39) studied the efficacy of cefloxacin and phage treatment in vitro using *Klebsiella pneumoniae* biofilm. Although antibiotic alone

has therapeutic efficacy, biofilm-forming bacteria include very resistant strains. Less resistant strains are formed after combined treatment. A highly synergistic effect of an anti-*Pseudomonas* phage cocktail combined with ciprofloxacin against *P. aeruginosa* was found (40). In conclusion, it has been shown in the present study that combined treatment eliminates biofilms and prevents the formation of resistant strains.

In our study, in the serum samples, the CRP level in the patient group was found significantly higher ($P < 0.05$) than in the treatment groups and it decreased after treatment. In the study by Majhi et al. (41), combined antibiotic treatment (levofloxacin and ceftriaxone) was applied in a pneumonia model caused by *Streptococcus pneumoniae* in BALB/c mice. The serum CRP level ($P < 0.05$) was decreased 24 h after treatment. The authors suggested that the reduction of bacterial burden in the lungs caused a decrease in CRP levels after treatment. These results support our findings.

Monitoring of lactate is an important marker in pneumonia. In our study, the lactate level in serum samples of the pneumonia group increased significantly ($P < 0.05$) compared with the control group; the high lactate level decreased after treatment. Lactate level in serum was elevated in patients with pneumonia and rapidly decreased to normal levels in 12–24 h of treatment (42).

In this study, a significant decrease ($P < 0.05$) in IL-6 levels in serum samples was observed in the treated groups compared with the patient group. Zimecki et al. (43) reported decreased IL-6 in serum after phage treatment (*S. aureus* A5/L) in a pneumonia mouse model. In their study, Matsuda et al. (44) produced peritoneal models in mice and treated them with lysine mutant phages. As a result of the treatment, there was a significant decrease in serum IL-6 level. A combination of levofloxacin and ceftriaxone was used in the treatment of lung inflammation in the mouse model, and similarly, a decrease in serum IL-6 levels was observed (41).

Debarbieux et al. (37) reported that levels of TNF-α and IL-6 in BAL samples were lowered as a result of phage treatment. Hung et al. (45) performed successful treatment with the φNK5 phage in a liver abscess caused by *Klebsiella pneumoniae*. After treatment, serum TNF-α and IL-6 levels were significantly decreased, and bacterial load was decreased in the blood. Although AST and ALT levels increased after infection, no change was observed in the phage treatment group. There was no significant difference in ALT and AST values. AST and ALT levels were elevated due to insufficient liver function after infection (45). *P. aeruginosa* may have had less load on the liver than in other studies because there was no significant increase in AST and ALT in our study.

Serum IL-6 levels were 338.6 pg/mL in the phage treatment group and 184.1 pg/mL in the control group. IL-6 levels were found statistically significantly higher than in phage serum (t-test, $P < 0.0001$) and the phage + meropenem group (t-test, $P < 0.007$). Endotoxins released from gram-negative bacteria after phage treatment may increase the production of TNF-α and IL-6 (46,47). High phage concentration and prolonged exposure to phages can increase the proinflammatory response.

Pulmonary inflammation can cause secondary infections in other organs such as the kidneys. Si et al. (48) developed a model of acute respiratory distress syndrome by injecting tracheal lipopolysaccharide into mice and investigated kidney damage as a secondary infection. Poly polymerase (PARP) binds to the ADP-ribose of target proteins, changes chromatin structure and transcription regimes, and increases DNA damage. Excessive amounts of active PARP may trigger an immunologic response. It also leads to NF-κB activation, which leads to an increase in inflammatory cytokines.

The mean TNF-α level in kidney samples of the pneumonia group was 756.8 ± 49.6 pg/mL. In the treatment groups, the TNF-α level was decreased (254 pg/mL) and there was a statistically significant difference between the control group and the pneumonia group ($P < 0.05$). The mean hyaluronan value of the pneumonia group was 152.7 ± 16.5 ng/mL and the mean value in the treatment groups dropped to 132.7 ng/mL. The serum creatinine level was also elevated in the study as an indication of renal dysfunction. This study might have proved that communication between the lungs and kidneys is related to proinflammatory cytokines.

Pulmonary infections can trigger proinflammatory cytokines such as IL-6, TNF-α, and IL-1β. Increased cytokines trigger insulin resistance in the liver. IL-6 and TNF-α most likely reduce the effect of intestinal insulin, reduce glucose uptake, and thus increase the level of blood glucose. Lung infections trigger liver inflammation because there is communication between the lung and the liver. In this study, the serum lactate level increased with infection. Increased lactate enters the liver tissue, triggering glucose production, which can increase serum glucose levels.

The greatest reduction in lung bacterial load was obtained by the combined use of the PhiKZ phage and meropenem. Elevated proinflammatory cytokines due to infection decreased through the reduced burden of bacterial load after treatment. The level of hyaluronan, a sign of lung injury in pneumonia, was significantly higher in the patient group. The increase in IL-6 and TNF-α levels in kidney tissue suggests that there was an interaction between the lungs and the kidneys. Elevation of serum lactate and glucose values suggests that the same interaction may occur with the liver. We believe that this work will encourage and guide new studies on phage therapy.

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