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Lymphatic Pump Treatment as an Adjunct to Antibiotics for Pneumonia in a Rat Model

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Background: Lymphatic pump treatment (LPT) is a technique used by osteopathic physicians as an adjunct to antibiotics for patients with respiratory tract infections, and previous studies have demonstrated that LPT reduces bacterial load in the lungs of rats with pneumonia. Currently, it is unknown whether LPT affects drug efficacy.

Objective: To determine whether the combination of antibiotics and LPT would reduce bacterial load in the lungs of rats with acute pneumonia.

Methods: Rats were infected intranasally with 5×10^7 colony-forming units (CFU) of *Streptococcus pneumoniae*. At 24, 48, and 72 hours after infection, the rats received no therapy (control), 4 minutes of sham therapy, or 4 minutes of LPT, followed by subcutaneous injection of 40 mg/kg of levofloxacin or sterile phosphate-buffered saline. At 48, 72, and 96 hours after infection, the spleens and lungs were collected, and *S pneumoniae* CFU were enumerated. Blood was analyzed for a complete blood cell count and leukocyte differential count.

Results: At 48 and 72 hours after infection, no statistically significant differences in pulmonary CFU were found between control, sham therapy, or LPT when phosphate-buffered saline was administered; however, the reduction in CFU was statistically significant in all rats given levofloxacin. The combination of sham therapy and levofloxacin decreased bacterial load at 72 and 96 hours after infection, and LPT and levofloxacin significantly reduced CFU compared with sham therapy and levofloxacin at both time points ($P<.05$). Colony-forming units were not detected in the spleens at any time. No statistically significant differences in hematologic findings between any treatment groups were found at any time point measured.

Conclusion: The results suggest that 3 applications of LPT induces an additional protective mechanism when combined with levofloxacin and support its use as an adjunctive therapy for the management of pneumonia; however, the mechanism responsible for this protection is unclear.

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Community-acquired pneumonia (CAP) accounts for more than 1 million hospital admissions each year worldwide.[1] Notwithstanding the costs of antibiotics for the management of CAP, hospital room and board is the single most expensive component of health care in the United States. The mean length of hospital stay for CAP is 5.6 days for patients aged 65 years or older and 4.5 days for younger patients,[2] making the cost of hospitalization for CAP more than $8 billion annually.[3] Shortening the length of hospital stay by 1 day could save more than $1 billion per year; therefore,

therapies that shorten hospitalization or allow outpatient management should be exploited.

Lymphatic pump treatment (LPT) comprises osteopathic manipulative medicine techniques that target the musculoskeletal system and enhance the flow of lymph through the lymphatic system.[4,5] Clinically, LPT is reported to increase vaccine-specific antibodies,[6,7] reduce the need for intravenous antibiotics,[8,9] protect against lower respiratory tract disease,[8-10] and shorten the duration of hospital stay in elderly patients with pneumonia.[9] To our knowledge, no reports have been published measuring the effects of LPT on the lymphatic system in humans, but animal studies have demonstrated that LPT yields significant ($P<.05$) increases in lymph flow and in the lymphatic concentration of leukocytes.[11-18] In these studies, LPT did not preferentially mobilize any specific immune cell population, but it significantly ($P<.05$) increased thoracic duct lymph flow and total leukocyte concentrations, resulting in an approximately 10-fold increase in the lymphatic flux.[12] Lymphatic pump treatment also significantly ($P<.05$) enhanced the lymphatic flux of inflammatory cytokines, chemokines, and reactive oxygen and nitrogen species in both thoracic and mesenteric lymph,[17,18] and it enhanced the uptake of protein from the tissue's interstitial space.[19] Collectively, these studies suggest that LPT can enhance the lymphatic and immune system responses, which may accelerate the clearance of pneumococcal bacteria.

A study published in 2010[15] used a rat model to investigate the effect of LPT on lymphatic function. Rats received LPT in a manner similar to that reported previously,[19] and lymph was collected from the cisterna chyli. Four minutes of LPT caused a statistically significant increase in lymph flow and leukocyte concentrations. These results were consistent with findings in dogs[11-13,16] and demonstrated the enhanced lymph flow and the lymphatic release of immune cells induced by LPT in a smaller animal.

In a later study,[14] rats were infected intranasally with *Streptococcus pneumoniae* and received sham therapy or LPT. The application of LPT once daily for 7 consecutive days reduced the concentration of bacteria in the lungs approximately 30-fold compared with sham therapy. In a follow-up study, Creasy et al[20] found that 3 applications once daily for 3 consecutive days of either thoracic or abdominal LPT were able to significantly ($P<.05$) reduce the numbers of pulmonary *S pneumoniae* colony-forming units (CFU) compared with control or sham therapy. These results suggest that LPT may protect against bacterial pneumonia by inhibiting bacterial growth in the lung; however, the mechanism responsible for this clearance is still under investigation.

To our knowledge, no study has been published to show that lymphatic treatments, such as LPT, affect drug efficacy. In the current study, we hypothesized that the combination of antibiotics and LPT would reduce the concentration of bacteria in the lungs of rats infected with *S pneumoniae*. Antibiotics are generally used to manage bacterial pneumonia and other infectious diseases; therefore, it is important to identify how complementary therapies, such as LPT, affect antibiotic efficacy.

Methods

Animals

The present study was approved by the Institutional Animal Care and Use Committee and conducted in accordance with the Guide for the Care and Use of Laboratory Animals.[21] Male inbred Fischer 344 rats, free of clinically evident signs of disease and weighing between 200 to 300 g, were used. The rats had indwelling jugular vein catheters, which remained in place for the duration of the study.

Infection

Anesthesia was delivered (30 mg/kg ketamine and 5 mg/kg xylazine), and the rats were intranasally inoculated with 5×10^7 *S pneumoniae* (ATCC 6301) CFU in 100 μL of phosphate-buffered saline (PBS). After infection, the rats were held vertically for a few seconds to allow for aspiration of the fluid. No substantial weight loss or any

other clinical signs of disease were observed in the animals during the study (data not shown). Therefore, "disease free" was defined as an absence of *S pneumoniae* in lung homogenates.

Leukocyte Enumeration

Blood samples were collected by means of cardiac puncture and drawn into EDTA-coated vacutainer blood test tubes. Total leukocytes and a differential leukocyte count were enumerated using an automatic hematology analyzer.

Bacterial Enumeration

S pneumoniae ATCC 6301 stocks were stored at −80°C in brain heart infusion broth containing 10% glycerol until use. Bacteria were cultured on trypticase soy agar with 5% sheep blood agar plates and incubated overnight at 37°C and 5% carbon dioxide. The bacteria were collected by washing the plates twice with sterile PBS and diluted to an optical density at 600 nm for infection. The CFU concentration in the suspension was determined retrospectively by serial dilution and plating on trypticase soy agar with 5% sheep blood agar plates. For enumeration of bacteria in pulmonary and spleen tissue, lungs and spleens were removed and homogenized separately for 25 seconds using a tissue homogenizer. Ten-fold (1:10 to 1:1,000,000) serial dilutions were made in a 96-well plate. Twenty microliters of each dilution were plated onto trypticase soy agar with 5% sheep blood plates in duplicates. The plates were incubated overnight at 37˚C with 5% carbon dioxide, and CFU were counted after 18 hours.

Levofloxacin

The sensitivity of the ATCC 6301 strain of *S pneumoniae* to levofloxacin was confirmed in vitro using the Kirby Bauer susceptibility test protocol (data not shown). Dosage studies were performed to determine the optimum concentration of levofloxacin to be used in vivo. Rats were intranasally infected with 5×10^7 CFU and received sterile PBS or 25, 40, or 50 mg/kg of levofloxacin resuspended in sterile PBS. At 24, 48, and 72 hours after infection, rats received no manual therapy (control), sham therapy, or LPT followed by subcutaneous injection of 40 mg/kg of levofloxacin or sterile PBS.

Interventions

Abdominal LPT was applied to rats as previously described.[14,15,20] Briefly, anesthetized (10 mg/kg propofol) rats were kept in a right lateral recumbent position. To perform LPT, the operator (A.S.) contacted the abdomen of the rat with the thumb on 1 side and index finger and middle finger on the other side of the medial sagittal plane. The fingers were placed bilaterally caudad to the ribs. Sufficient pressure was exerted medially and cranially to compress the lower ribs until substantial resistance was met against the diaphragm, then the pressure was released. Compressions were administered at a rate of approximately 1 per second for the duration of the 4 minutes of treatment. During sham therapy, rats were anesthetized and the operator contacted the abdomen of the rat for 4 minutes in a manner similar to LPT; however, no compressions were applied. This procedure was designed by H.H.K. to simulate the LPT used in humans.[15]

Data Collection

At 48, 72, and 96 hours after infection, the rats were euthanized in accordance with the American Veterinary Medical Association guidelines. The lungs and spleens were collected and the concentrations of *S pneumoniae* were measured in each tissue. Blood samples were analyzed at 0, 24, 48, 72, and 96 hours for a complete blood cell count and a leukocyte differential count. Details of the experimental design are outlined in *Figure 1*.

Statistical Analysis

A power analysis performed using the SD from our preliminary studies confirmed that 8 rats per group were sufficient for detecting differences between the means of the experiments, with a power of 0.90. Data on CFU

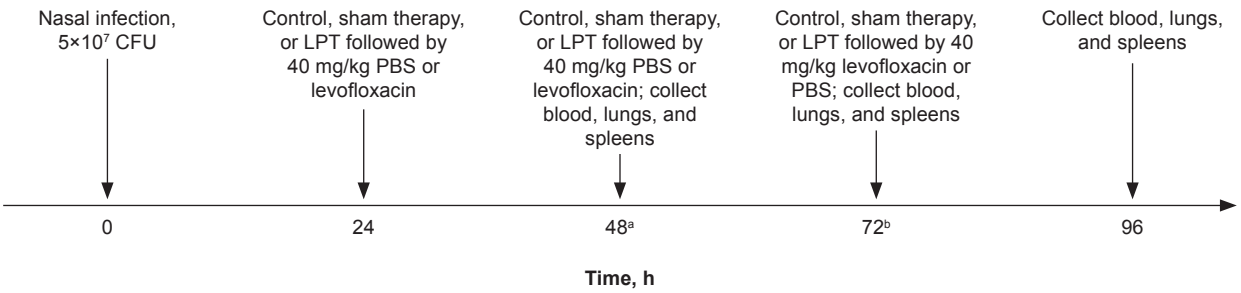

Figure 1.
Experimental protocol. On day 0, rats were intranasally infected with 5×10^7 *Streptococcus pneumoniae* colony-forming units (CFU) and randomly assigned into control (n=8), sham therapy (n=8), or lymphatic pump treatment (LPT) (n=8) groups. At 24, 48, and 72 hours after infection, rats received control, sham therapy, or LPT followed by subcutaneous injection of 40 mg/kg of levofloxacin or sterile phosphate-buffered saline (PBS). *S pneumoniae* CFU were enumerated at 48, 72, and 96 hours after infection. [a]LPT or sham therapy was administered at 24 and 48 hours after infection, and rats whose lungs and spleens were collected at 48 hours did not receive intervention at 48 hours. [b]LPT or sham therapy was administered at 24, 48, and 72 hours after infection, and rats whose lungs and spleens were collected at 72 hours did not receive intervention at 72 hours.

were logarithmically transformed before analysis. Data from control, sham therapy, or LPT were analyzed by an analysis of variance followed by a Tukey post hoc test using Graphpad Prism version 5.0 for Windows (GraphPad Software). Differences among mean values with $P<.05$ were considered statistically significant.

Results

Eight rats were in each group, as follows: control, sham therapy, and LPT, and euthanized at 48 hours; control, sham therapy, and LPT, and euthanized at 72 hours; and control, sham therapy, and LPT, and euthanized at 96 hours. In addition, each of the groups by euthanization time received either PBS or levofloxacin before euthanization. Therefore, a total of 144 rats were used in the present study. Data are presented as mean (SD) unless otherwise indicated.

LPT Protects Against Bacterial Pneumonia in a Dose-Dependent Manner

Three applications of LPT significantly reduced the concentration of pulmonary bacteria compared with control and sham therapy ($P<.05$) (*Figure 2*). Additionally, rats did not have any CFU in their spleens (data not shown).

LPT as an Adjunctive Therapy for Levofloxacin

Levofloxacin is a fluoroquinolone that is commonly used as a first choice for the management of CAP.[3,22] Levofloxacin reduced *S pneumoniae* bacteria in a dose-dependent manner over time (*Figure 3*). At 72 and 96 hours after infection, both 40 mg/kg and 50 mg/kg of levofloxacin significantly reduced CFU compared with PBS and 25 mg/kg of levofloxacin ($P<.05$). At 96 hours after infection, no CFU were detectable in the group receiving 50 mg/kg of levofloxacin. Considering that no CFU were detectable in the 50-mg/kg levofloxacin group at 96 hours after infection and that the protective effect of LPT was not detectable until 96 hours after infection (*Figure 2*), we chose 40 mg/kg of levofloxacin as the treatment dose for this study.

At 48 hours after infection, no significant differences were noted in pulmonary CFU concentration between the control plus PBS (1.0×10^7 [3.3×10^6]), sham therapy plus PBS (3.6×10^6 [6.3×10^5]), and LPT plus PBS

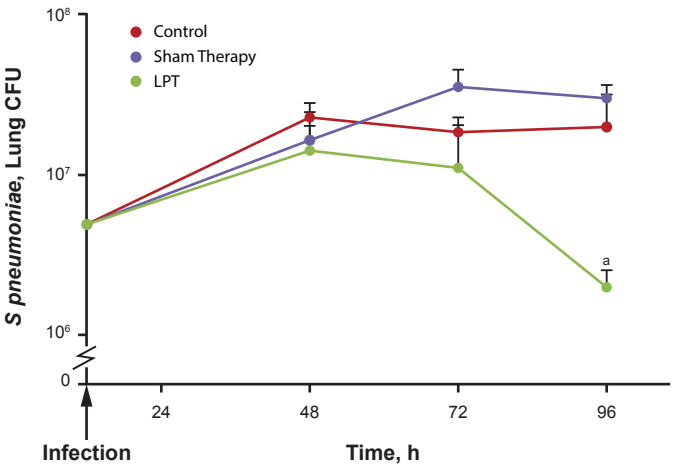

Figure 2.
Lymphatic pump treatment (LPT) protects against bacterial pneumonia. *Streptococcus pneumoniae* colony-forming units (CFU) were enumerated at 48, 72, and 96 hours after infection in a rat model. [a]*P*<.05 compared with control and sham therapy.

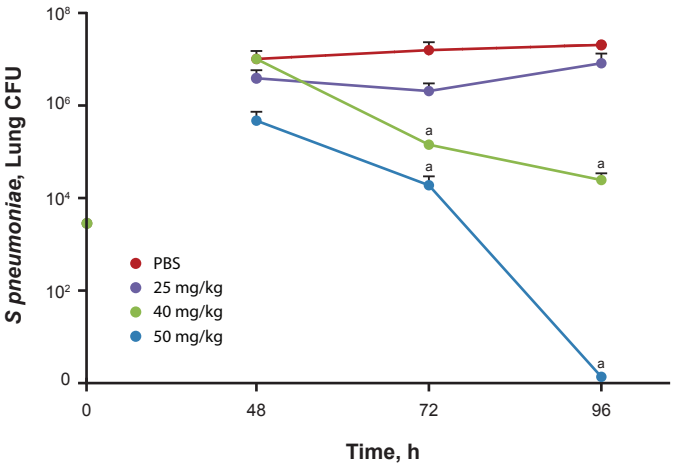

Figure 3.
Levofloxacin dosing. On day 0, rats were nasally infected with 5×10^7 *Streptococcus pneumoniae* colony-forming units (CFU) and received phosphate-buffered saline (PBS) and 25 mg/kg (n=5), 40 mg/kg (n=5), or 50 mg/kg (n=5) of levofloxacin at 24, 48, and 72 hours after infection. *S pneumoniae* CFU were enumerated at 48, 72, and 96 hours after infection. **P*<.05 compared with sham therapy and control.

(2.1×10^6 [5.8×10^5]) groups (*P*>.05). The addition of levofloxacin significantly reduced *S pneumoniae* CFU in the lungs compared with all PBS groups (*P*<.05); however, no statistical differences were found between the control plus levofloxacin (5.0×10^5 [3×10^5]), sham therapy plus levofloxacin (4.1×10^4 [1.4×10^4]), and LPT plus levofloxacin (2.7×10^4 [1.4×10^4]) groups. Furthermore, CFU were not detected in spleens. These results are summarized in *Figure 4A*.

Similarly, at 72 hours after infection, no significant differences were noted in pulmonary CFU between the control plus PBS (5.0×10^6 [5.8×10^5]), sham therapy plus PBS (3.2×10^6 [7.6×10^5]), or LPT plus PBS (2.6×10^6 [6.6×10^5]) groups (*P*>.05). As expected, the addition of levofloxacin significantly reduced *S pneumoniae* CFU in the lungs compared with all of the PBS groups (*P*<.05). There were significantly fewer bacteria in sham therapy plus levofloxacin (1.9×10^4 [1.0×10^4]) and LPT plus levofloxacin (4.7×10^3 [2.2×10^3]) groups compared with the control plus levofloxacin group (3.5×10^4 [9.0×10^3]) (*P*<.05). Furthermore, CFU were not detected in spleens. These results are summarized in *Figure 4B*.

At 96 hours after infection, the number of CFU in the LPT plus PBS group (0.9×10^6 [0.3×10^6]) were significantly reduced compared with the control plus PBS group (4.2×10^6 [1.0×10^6]) (*P*<.05). Although the addition of levofloxacin significantly reduced *S pneumoniae* CFU in the lungs compared with all PBS groups (*P*<.05), the administration of LPT plus levofloxacin (531 [261]) significantly reduced bacterial load compared with all intervention groups (*P*<.05) (*Figure 4C*). Furthermore, CFU were not detected in spleens (data not shown),

At 96 hours after infection, all of the rats in the control plus PBS and sham therapy plus PBS groups had disease (8 of 8 rats had *S pneumoniae* in their lungs). Rats in the control plus levofloxacin (2 of 8), sham therapy plus levofloxacin (3 of 8), and LPT plus PBS (1 of 8) groups were moderately disease free; however, more than half (5 of 8) of the rats in the LPT plus levofloxacin group were disease free. These results are summarized in *Table 1*.

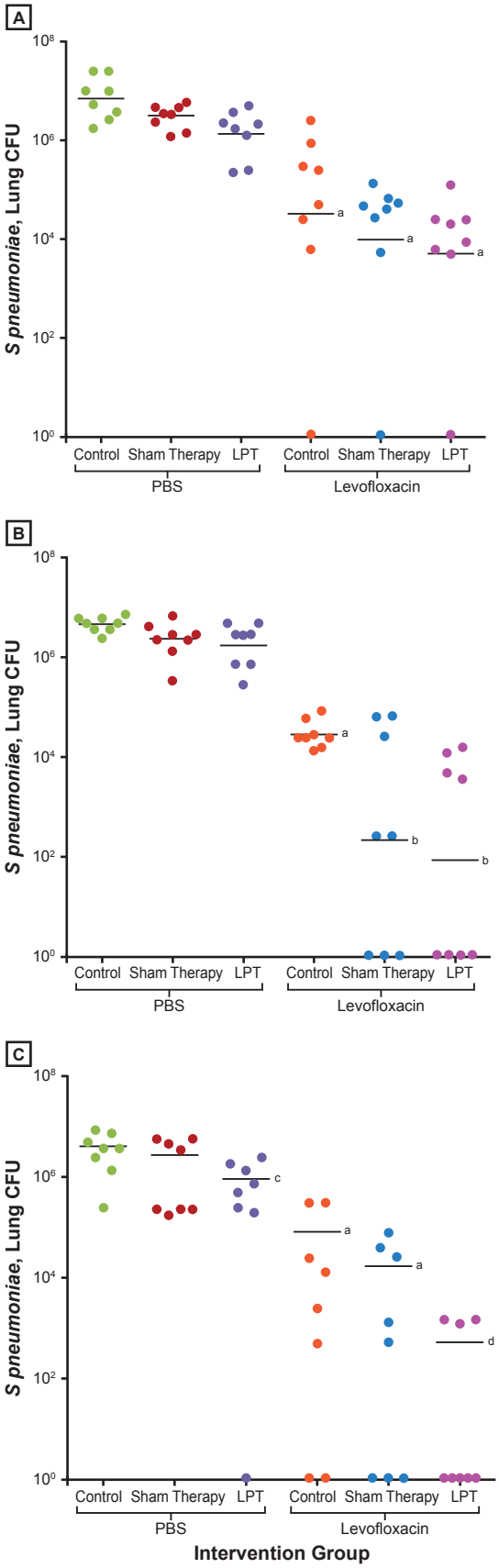

Figure 4.
Pulmonary *Streptococcus pneumoniae* levels (A) 48, (B) 72, and (C) 96 hours after infection. At each time point, rats were euthanized and colony-forming units (CFU) were enumerated. During intervention sessions at 24 hours; 24 and 48 hours; and at 24, 48, and 72 hours, respectively, rats received no therapy (control), sham therapy, or lymphatic pump treatment (LPT) followed by subcutaneous injection of 40 mg/kg levofloxacin or sterile phosphate-buffered saline (PBS). [a]*P*<.05 compared with respective PBS injection groups. [b]*P*<.05 compared with control + levofloxacin groups. [c]*P*<.05 compared with control + PBS injection groups [d]*P*<.05 compared with all other intervention groups.

Table 1.
Disease Status 96 Hours After Infection With *Streptococcus pneumoniae* in a Rat Model

Intervention	Disease Free, %
LPT + PBS	13
Sham therapy + PBS	0
Control[a] + PBS	0
LPT + levofloxacin	63[b]
Sham therapy + levofloxacin	38
Control + levofloxacin	25

[a] The control group did not receive any manual therapy.
[b] Exceeded the upper limit by analysis of means for proportions.

Abbreviations: LPT, lymphatic pump treatment; PBS, phosphate-buffered saline.

Effect of LPT on Hematologic Factors

Hematologic factors were within the normal range for rats at all time points measured. The differences in levels between baseline and 24 hours after infection are summarized in *Table 2*. At 48, 72, and 96 hours after infection, no statistically significant differences were found in the complete blood cell count or leukocyte differential count between any of the intervention groups (*Table 3*, *Table 4*, and *Table 5*).

Table 2.
Hematologic Test Results at Baseline and 24 h After Infection With *Streptococcus pneumoniae*[a] in a Rat Model

Test Result	Baseline[b]	24 h[b]
WBC, 10^6 cells/mL	5.3 (0.4)	4.6 (0.2)
Neutrophil, 10^6 cells/mL	1.9 (0.2)	1.8 (0.1)
Lymphocyte, 10^6 cells/mL	3.0 (0.2)	2.5 (0.1)
Monocyte, 10^6 cells/mL	0.4 (0.04)	0.2 (0.01)
Eosinophil, 10^6 cells/mL	0.01 (0.007)	0.02 (0.008)
Basophil, 10^6 cells/mL	0.01 (0.005)	0.01 (0.005)
RBC, 10^6 cells/mL	8.4 (0.1)	9.0 (0.1)
Hemoglobin, g/dL	13.0 (0.3)	14.5 (0.2)
Hematocrit, %	48.7 (0.8)	52.2 (0.6)
MCV, fL	58.4 (0.2)	58.1 (0.3)
MCH, pg	15.5 (0.1)	16.2 (0.2)
MCHC, g/dL	26.6 (0.2)	27.8 (0.3)
RDW, %	15.8 (0.1)	14.1 (0.1)
Platelet, 10^8 cells/mL	7.5 (1.0)	4.9 (0.2)
MPV, fL	5.8 (0.1)	6.3 (0.1)

[a] 5×10^7 colony-forming units of *S pneumoniae*.
[b] Data were analyzed by an analysis of variance, followed by a Tukey post hoc test, and are presented as mean (SD).

Abbreviations: MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; RBC, red blood cell; RDW, red blood cell distribution width; WBC, white blood cell.

Discussion

The current study found that when applied once daily for 3 consecutive days, the combination of levofloxacin and LPT significantly reduced the number of *S pneumoniae* bacteria in the lungs compared with levofloxacin or LPT alone ($P<.05$). The multicenter osteopathic study in the elderly (MOPSE), a double-blinded randomized controlled trial, measured the efficacy of osteopathic manipulation as an adjunctive treatment for hospitalized elderly patients with pneumonia.[8,9] Within 24 hours of admission, patients were randomly assigned into conventional care (including antibiotics), conventional care plus light touch, or conventional care plus OMT (including LPT). An intention-to-treat analysis found no statistically significant difference between the groups for any outcome; however, per-protocol analysis found that OMT plus conventional care reduced the length of hospital stay and the duration of intravenous antibiotic use and decreased the incidence of respiratory failure or death compared with the conventional care group alone.[9] Although the results from the MOPSE study support the use of OMT as an adjunctive therapy for the treatment of patients with pneumonia, the mechanism responsible for this protection is not clear. The results from the current study support the findings from the MOPSE study and suggest that LPT may protect against pneumonia by removing bacteria from the lungs and enhancing the efficacy of antibiotics.

In the current study, the combination of sham therapy and levofloxacin decreased bacterial load at 72 and 96 hours after infection. It is possible that the propofol anesthesia administered during sham therapy and LPT enhanced pulmonary protection. Propofol has been shown to protect against acute lung injury in rats by abrogating the microvascular leakage of water and protein in the lungs[23] and suppressing inflammatory-mediated injuries.[24] Also, light touch may have enhanced protection against infection, although this mechanism is less clear. Importantly, LPT plus levofloxacin cleared more

Table 3.
Hematologic Test Results 48 h After Infection With *Streptococcus pneumoniae*[a] in a Rat Model

	PBS[b]			Levofloxacin[b]		
Test Result	**Control**	**Sham Therapy**	**LPT**	**Control**	**Sham Therapy**	**LPT**
WBC, 10^6 cells/mL	3.3 (0.4)	3.2 (0.6)	3.2 (0.5)	3.8 (0.4)	4.0 (0.4)	4.2 (0.2)
Neutrophil, 10^6 cells/mL	1.2 (0.1)	1.3 (0.2)	1.3 (0.2)	1.3 (0.1)	1.5 (0.2)	1.5 (0.07)
Lymphocyte, 10^6 cells/mL	2.0 (0.3)	1.8 (0.3)	1.7 (0.2)	2.3 (0.3)	2.3 (0.2)	2.5 (0.2)
Monocyte, 10^6 cells/mL	0.1 (0.03)	0.1 (0.02)	0.1 (0.03)	0.1 (0.02)	0.2 (0.02)	0.2 (0.02)
Eosinophil, 10^5 cells/mL	0.03 (0.02)	0.06 (0.03)	0.1 (0.05)	0.08 (0.04)	0.1 (0.06)	0.1 (0.04)
Basophil, 10^5 cells/mL	0.02 (0.02)	0.01 (0.01)	0.04 (0.02)	0.03 (0.02)	0.08 (0.04)	0.08 (0.04)
RBC, 10^9 cells/mL	8.4 (0.3)	8.1 (0.2)	8.0 (0.5)	8.3 (0.4)	8.3 (0.3)	8.0 (0.3)
Hemoglobin, g/dL	13.5 (0.5)	13.2 (0.4)	12.7 (0.8)	12.8 (0.7)	12.8 (0.5)	13.5 (0.3)
Hematocrit, %	49.8 (1.9)	48.2 (1.5)	46.9 (2.9)	47.4 (2.6)	46.9 (1.9)	49.1 (1.0)
MCV, fL	59.1 (0.5)	59.3 (0.6)	58.8 (0.4)	57.1 (0.6)	56.5 (0.4)	58.1 (1.1)
MCH, pg	16.0 (0.1)	16.2 (0.2)	15.9 (0.1)	15.5 (0.3)	15.4 (0.1)	15.7 (0.3)
MCHC, g/dL	27.1 (0.3)	27.1 (0.2)	27.1 (0.2)	27.1 (0.3)	31.0 (3.9)	27.6 (0.4)
RDW, %	13.9 (0.1)	13.9 (0.1)	13.6 (0.2)	14.6 (0.2)	14.6 (0.2)	14.8 (0.3)
Platelet, 10^8 cells/mL	2.8 (0.9)	1.8 (0.7)	2.0 (0.9)	3.6 (0.7)	4.3 (0.6)	4.8 (0.6)
MPV, fL	8.6 (1.1)	8.8 (0.9)	7.3 (0.5)	7.2 (0.5)	6.9 (0.6)	6.4 (0.1)

[a] 5×10^7 colony-forming units of *S pneumoniae*. Eight rats were in each control, sham therapy, and lymphatic pump treatment (LPT) group.
[b] Data were analyzed by an analysis of variance, followed by a Tukey post hoc test, and are presented as mean (SD).

Abbreviations: MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; PBS, phosphate-buffered saline; RBC, red blood cell; RDW, red blood cell distribution width; WBC, white blood cell.

bacteria compared with sham therapy plus levofloxacin at both time points, suggesting that LPT plus levofloxacin induces an additional protective mechanism compared with sham therapy plus levofloxacin.

We did not identify the mechanism by which LPT reduced bacteria in the lungs, which is a limitation. It is possible that LPT may enhance the delivery of levofloxacin to the lung. In support, the pharmacodynamic pattern of levofloxacin is linked to the clinical outcome,[22] and enhancing the delivery of levofloxacin to the lung via LPT would support its use as an adjunctive therapy. Furthermore, LPT has been shown to enhance the uptake of protein from the interstitial space and its transport to the blood[19]; therefore, LPT may have increased protection against *S pneumoniae* by enhancing the uptake of levofloxacin from the tissue and its delivery to the blood. In the current study, levofloxacin was administered immediately after sham therapy or LPT; therefore, it is important to mention that the protective effect of LPT may have been greatly enhanced had levofloxacin been administered before LPT. Nonetheless, further experimentation is necessary to identify the mechanisms by which LPT acts as an adjunctive therapy in this research model.

Table 4.
Hematologic Test Results 72 h After Infection With *Streptococcus pneumoniae*[a] in a Rat Model

	PBS[b]			Levofloxacin[b]		
Test Result	**Control**	**Sham Therapy**	**LPT**	**Control**	**Sham Therapy**	**LPT**
WBC, 10^6 cells/mL	5.3 (0.4)	5.5 (0.4)	5.3 (0.3)	4.1 (0.5)	4.0 (0.4)	3.9 (.05)
Neutrophil, 10^6 cells/mL	1.9 (0.2)	2.1 (0.2)	2.1 (0.2)	1.2 (0.09)	1.4 (0.1)	1.3 (0.2)
Lymphocyte, 10^6 cells/mL	3.1 (0.2)	3.2 (0.2)	2.9 (0.2)	2.6 (0.3)	2.4 (0.3)	2.4 (0.3)
Monocyte, 10^6 cells/mL	0.2 (0.03)	0.3 (0.02)	0.2 (0.03)	0.2 (0.03)	0.2 (0.03)	0.2 (0.03)
Eosinophil, 10^5 cells/mL	0.2 (0.1)	0.08 (0.04)	0.2 (0.08)	0.1 (0.02)	0.06 (0.02)	0.1 (0.06)
Basophil, 10^5 cells/mL	0.1 (0.06)	0.05 (0.04)	0.1 (0.05)	0.04 (0.02)	0.03 (0.02)	0.05 (0.03)
RBC, 10^9 cells/mL	9.0 (0.2)	8.7 (0.1)	8.7 (0.1)	8.5 (0.3)	8.3 (0.4)	7.8 (0.4)
Hemoglobin, g/dL	13.9 (0.3)	13.8 (0.2)	13.6 (0.3)	13.0 (0.3)	12.8 (0.6)	12.2 (0.6)
Hematocrit, %	51.9 (0.9)	51.1 (0.7)	50.4 (0.7)	48.7 (1.2)	47.2 (2.3)	44.4 (2.2)
MCV, fL	57.9 (0.3)	58.4 (0.4)	58.1 (0.3)	57.5 (1.1)	56.5 (0.3)	57.3 (0.4)
MCH, pg	15.4 (0.2)	15.8 (0.1)	15.7 (0.2)	15.4 (0.2)	15.0 (0.3)	15.8 (0.5)
MCHC, g/dL	26.7 (0.2)	27.0 (0.2)	27.0 (0.4)	26.8 (0.2)	27.1 (0.3)	27.7 (1.0)
RDW, %	14.2 (0.1)	14.1 (0.1)	14.1 (0.1)	16.1 (1.3)	14.9 (0.2)	14.7 (0.2)
Platelet, 10^8 cells/mL	4.6 (0.8)	6.4 (0.3)	5.9 (0.7)	4.8 (0.5)	4.4 (0.8)	5.1 (0.7)
MPV, fL	6.6 (0.3)	6.2 (0.2)	6.5 (0.2)	6.5 (0.1)	7.0 (0.4)	6.9 (0.6)

[a] 5×10^7 colony-forming units of *S pneumoniae*. Eight rats were in each control, sham therapy, and lymphatic pump treatment (LPT) group.
[b] Data were analyzed by an analysis of variance, followed by a Tukey post hoc test, and are presented as mean (SD).

Abbreviations: MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; PBS, phosphate-buffered saline; RBC, red blood cell; RDW, red blood cell distribution width; WBC, white blood cell.

It is also possible that LPT enhanced pulmonary immunity. Studies in 2010[12] and 2012[17] demonstrated that LPT enhanced thoracic and mesenteric lymph flow, mobilized leukocytes from the gastrointestinal lymphoid tissues into lymph circulation,[12] and increased the lymphatic flux of leukocytes, cytokines, and reactive oxygen and nitrogen species.[17,18] Collectively, these studies suggest that LPT can stimulate the lymphatic and immune systems, which may accelerate the removal of pneumonia by the immune system. In support, lymph can activate leukocytes,[25] increase endothelial cell permeability,[26,27] and redistribute leukocytes, cytokines, and chemokines to the lung.[28] If the lung is infected, measures that enhance lymph output, such as LPT, may accelerate the immune-mediated clearance of the microbes; however, whether LPT enhances this process is unknown.

Conclusion

The combination of levofloxacin and LPT significantly reduced the concentration of pulmonary bacteria compared with LPT or levofloxacin alone ($P<.05$). To our knowledge, this study is the first to demonstrate that

Table 5.
Hematologic Test Results at Baseline and 96 h After Infection With *Streptococcus pneumoniae*[a] in a Rat Model

	PBS[b]			Levofloxacin[b]		
Test Result	**Control**	**Sham Therapy**	**LPT**	**Control**	**Sham Therapy**	**LPT**
WBC, 10^6 cells/mL	5.8 (0.4)	5.1 (0.4)	5.2 (0.4)	4.5 (0.4)	4.5 (0.3)	4.7 (0.4)
Neutrophil, 10^6 cells/mL	2.5 (0.2)	2.0 (0.2)	1.9 (0.2)	1.7 (0.2)	1.8 (0.2)	1.9 (0.2)
Lymphocyte, 10^6 cells/mL	3.0 (0.2)	2.8 (0.2)	3.0 (0.3)	2.5 (0.3)	2.4 (0.2)	2.6 (0.2)
Monocyte, 10^6 cells/mL	0.2 (0.03)	0.3 (0.02)	0.3 (0.04)	0.3 (0.03)	0.3 (0.03)	0.3 (0.03)
Eosinophil, 10^5 cells/mL	0.01 (0.003)	0.02 (0.005)	0.006 (0.003)	0.01 (0.01)	0.006 (0.002)	0.006 (0.003)
Basophil, 10^5 cells/mL	0.004 (0.002)	0.01 (0.003)	0.004 (0.002)	0.008 (0.006)	0.005 (0.002)	0.004 (0.002)
RBC, 10^9 cells/mL	7.8 (1.1)	7.9 (0.4)	7.9 (1.1)	8.4 (0.4)	7.8 (0.3)	8.0 (0.4)
Hemoglobin, g/dL	14.1 (0.1)	12.5 (0.7)	13.7 (0.3)	13.3 (0.7)	12.3 (0.4)	12.5 (0.7)
Hematocrit, %	51.0 (0.6)	45.7 (2.4)	51.5 (1.0)	47.9 (2.2)	44.5 (1.4)	45.1 (2.1)
MCV, fL	57.4 (0.4)	57.9 (0.4)	59.0 (0.9)	56.7 (0.2)	56.9 (0.2)	56.9 (0.3)
MCH, pg	15.9 (0.1)	15.8 (0.2)	15.8 (0.1)	15.7 (0.2)	15.6 (0.2)	15.7 (0.2)
MCHC, g/dL	27.6 (0.3)	27.3 (0.3)	27.3 (0.2)	27.6 (0.3)	27.5 (0.4)	27.5 (0.4)
RDW, %	14.7 (0.3)	14.6 (0.2)	14.9 (0.3)	15.7 (0.2)	15.4 (0.2)	15.2 (0.2)
Platelet, 10^8 cells/mL	7.3 (0.8)	6.2 (0.7)	9.0 (1.9)	7.0 (0.8)	6.6 (0.7)	7.6 (0.5)
MPV, fL	6.1 (0.1)	6.3 (0.1)	6.0 (0.1)	6.1 (0.1)	6.2 (0.1)	5.8 (0.1)

[a] 5×10^7 colony-forming units of *S pneumoniae*. Eight rats were in each control, sham therapy, and lymphatic pump treatment (LPT) group.
[b] Data were analyzed by an analysis of variance, followed by a Tukey post hoc test, and are presented as mean (SD).

Abbreviations: MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; PBS, phosphate-buffered saline; RBC, red blood cell; RDW, red blood cell distribution width; WBC, white blood cell.

LPT acts synergistically with antibiotics for the treatment of patients with pneumonia. Translational studies such as the current study are crucial to identify the mechanisms by which LPT protects against infectious disease and to support its clinical use in patients with pneumonia. Once these mechanisms are understood, LPT can be optimally applied to patients with pneumonia, which may substantially reduce morbidity, mortality, and the cost of hospitalization.

Author Contributions

All authors provided substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; all authors drafted the article or revised it critically for important intellectual content; Dr Hodge gave final approval of the version of the article to be published; and all authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

(*continued*)

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