Efficacy of Lysophosphatidylcholine in Combination with Antimicrobial Agents against Acinetobacter baumannii in Experimental Murine Peritoneal Sepsis and Pneumonia Models


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Immune response stimulation to prevent infection progression may be an adjuvant to antimicrobial treatment. Lysophosphatidylcholine (LPC) is an immunomodulator involved in immune cell recruitment and activation. In this study, we aimed to evaluate the efficacy of LPC in combination with colistin, tigecycline, or imipenem in experimental murine models of peritoneal sepsis and pneumonia. We used Acinetobacter baumannii strain Ab9, which is susceptible to colistin, tigecycline, and imipenem, and multidrug-resistant strain Ab186, which is susceptible to colistin and resistant to tigecycline and imipenem. Pharmacokinetic and pharmacodynamic parameters for colistin, tigecycline, and imipenem and the 100% minimal lethal dose (MLD100) were determined for both strains. The therapeutic efficacies of LPC, colistin (60 mg/kg body weight/day), tigecycline (10 mg/kg/day), and imipenem (180 mg/kg/day), alone or in combination, were assessed against Ab9 and Ab186 at the MLD100 in murine peritoneal sepsis and pneumonia models. The levels of pro- and anti-inflammatory cytokines, i.e., tumor necrosis factor alpha (TNF-α) and interleukin-10 (IL-10), were determined by enzyme-linked immunosorbent assay (ELISA) for the same experimental models after inoculating mice with the MLD of both strains. LPC in combination with colistin, tigecycline, or imipenem markedly enhanced the bacterial clearance of Ab9 and Ab186 from the spleen and lungs and reduced bacteremia and mouse mortality rates (P < 0.05) compared with those for colistin, tigecycline, and imipenem monotherapies. Moreover, at 4 h post-bacterial infection, Ab9 induced higher TNF-α and lower IL-10 levels than those with Ab186 (4 μg/ml versus 3 μg/ml [P < 0.05] and 2 μg/ml versus 3.4 μg/ml [P < 0.05], respectively). LPC treatment combined with colistin, tigecycline, or imipenem modestly reduced the severity of infection by A. baumannii strains with different resistance phenotypes compared to LPC monotherapy in both experimental models.

Acinetobacter baumannii is a Gram-negative cocccobacillus with high clinical relevance due to the different severe nosocomial infections that it causes, mainly in intensive care units, and its capacity to develop resistance to most of the antimicrobial agents used in clinical practice (1).

A multidrug resistance pattern is commonly observed for A. baumannii isolates, raising the threat of impossible-to-treat infections (2). These multidrug-resistant (MDR) isolates are generally susceptible to polymyxins (colistin and polymyxin B) and resistant to imipenem and tigecycline (3, 4). The limited antimicrobial alternatives for the treatment of severe infections by MDR A. baumannii make the search for other therapeutic options urgent. Polymyxins have been used as a last resort to treat infections by MDR A. baumannii. In humans, suboptimal and optimal doses of colistin to treat ventilator-associated pneumonia due to MDR A. baumannii prevent mortality in only 38.1% and 62.5% of cases, respectively (5, 6). Recently, in a clinical trial at our hospital, treatment with the optimized dose of colistin, 3 million units (MU)/8 h, prevented mortality in only 50% of patients (4 of 8 patients) with A. baumannii infection (unpublished data). Moreover, the use of colistin to treat infections with A. baumannii clinical isolates sensitive to it protected only 33 to 40% of mice from mortality (7, 8).

Lysophosphatidylcholine (LPC) is a major component of phospholipids involved in the recruitment and stimulation of immune cells and the elimination of dead eukaryotic and prokaryotic cells during infection (9–13). We previously successfully demonstrated the efficacy of LPC as a preemptive treatment in murine peritoneal sepsis and pneumonia experimental models of infection caused by susceptible A. baumannii strains (14). From this study, we suggested the clinical application of LPC as an adjuvant pretreatment in combination with antimicrobial agents for the treatment of infections by A. baumannii, including MDR strains (14).

Currently, there are no data regarding the efficacy of LPC in combination with antimicrobials, such as colistin, tigecycline, and imipenem, all of which are commonly used in severe A. baumannii infections. Thus, the aim of this study was to evaluate the efficacy...
of LPC in combination with these antibiotics in a murine experimental model of peritoneal sepsis caused by two clinical isolates of *A. baumannii*: one susceptible to colistin, tigecycline, and imipenem and the other susceptible only to colistin and resistant to tigecycline and imipenem.

**MATERIALS AND METHODS**

**Bacterial strains.** An *A. baumannii* clinical strain (Ab9) susceptible to colistin, tigecycline, and imipenem, isolated from a wound surgical excudate, and an MDR *A. baumannii* clinical strain (Ab186) susceptible to colistin and resistant to tigecycline and imipenem, isolated from blood cultures, were used for this study, in addition to a reference strain (ATCC 19798) isolated from an infant with fatal meningitis (15). The Ab9 and Ab186 strains were from the REIPI-GEIH 2010 collection (4) and were of types ST297 and ST2 (international clone II), respectively.

**Antimicrobial agents and reagents.** For the *in vitro* assays, standard laboratory powders of the following antimicrobials were used: colistin (Sigma, Spain), tigecycline (Sequoia Research Products Ltd., United Kingdom), and imipenem (Sigma, Spain). For the *in vivo* experiments, clinical formulations of the following antimicrobials were used: colistin methanesulfonate (CMS) (G.E.S., Spain), tigecycline (Pfizer, Spain), and imipenem (Merck Sharp & Dohme, Spain). The anesthetic was 5% (wt/vol) sodium thiopental administered intraperitoneally (i.p.) (B. Braun Medical S.A., Spain).

**In vitro susceptibility testing.** MICs were determined by broth microdilution assay according to standard CLSI recommendations (16), as previously described (17). *Escherichia coli* ATCC 25922 was used as a control strain.

**Animals.** Immunocompetent C57BL/6 female mice weighing 18 to 20 g (Production and Experimentation Animal Center, University of Seville, Seville, Spain) were used. Animals were housed in regulation boxes and given free access to food and water. This study was carried out in strict accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals* (18). The protocol was approved by the Committee on the Ethics of Animal Experiments of the University Hospital of Virgen del Rocio of Seville, Spain (approval 2014PI/014).

**Antimicrobial pharmacokinetic (PK) and pharmacodynamic (PD) parameters.** Serum antimicrobial concentrations were determined for groups of 30 healthy mice following single doses of i.p. colistin methanesulfonate (20 mg/kg of body weight), subcutaneous (s.c.) tigecycline (5 mg/kg), or intramuscular (i.m.) imipenem (30 mg/kg).

For sets of three animals 0, 5, 10, 15, 30, 60, 90, 120, 240, and 1,440 min after drug administration, blood samples were obtained from anesthetized mice via the periorbital plexus. Concentrations of colistins A and B and their prodrugs, colistin methanesulfonate A and colistin methanesulfonate B, as well as tigecycline and imipenem, were measured using high-pressure liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) (19–21). The maximum concentration of drug in serum (*Cmax*) for colistin, the area under the concentration–time curve from 0 h to 24 h (*AUCC0–24*), the *AUCC0–∞* for the free, unbound fraction of drug (*AUUC0–24*), the half-life (*t1/2*), the cumulative percentage of a 24-h period that the drug concentration exceeds the MIC under steady-state pharmacokinetic conditions (% *TMMC*), the AUC over 24 h in the steady state divided by the MIC for the free, unbound fraction of drug (*AUC/MIC*), and the AUC/MIC value were obtained by a computer-assisted method (22). Final dosing for the *in vivo* efficacy experiments was adjusted to achieve values for the pharmacodynamic parameters *AUC/MIC* (for free colistin) and AUC/MIC (for total tigecycline) similar to the ranges (17.5 to 22.5 and 18.5 to 37) for colistin and tigecycline, respectively, reported in previous studies as necessary to reduce the bacterial burden in lungs by 2 log (for colistin treatment) and 3 log (for tigecycline treatment) in experimental models of pneumonia caused by *A. baumannii*, *E. coli*, and *Klebsiella pneumoniae* (23, 24). Final dosing of total imipenem was adjusted to achieve a % *TMMC* of at least 40% of the dosing interval (25).

Experimental murine model of peritoneal sepsis. A previously characterized murine model of peritoneal sepsis caused by *A. baumannii* was used (14). Briefly, animals were inoculated i.p. with 0.5 ml of the 100% minimal lethal dose (MLD<sub>100</sub> of the Ab9 or Ab186 strain, mixed 1:1 with 10% porcine mucin (Sigma, Spain). The MLD<sub>100</sub> the 50% lethal dose (LD<sub>50</sub>), and the LD<sub>0</sub>, the maximum inoculum resulting in no mortality, were determined by inoculating groups of 6 mice i.p. with decreasing concentrations of *A. baumannii*, from 8.78 to 2.3 log CFU/ml for the Ab9 strain and from 8.59 to 2.4 log CFU/ml for the Ab186 strain, and monitoring the survival of the mice for 7 days. LD<sub>0</sub> values for the strains used in challenge studies were determined using the Probit method. LPC therapy was administered as a pretreatment 1 h before bacterial inoculation, and antimicrobial therapy was initiated 4 h after bacterial inoculation.

Groups of 15 mice were randomly ascribed to the following groups: (i) controls (without treatment), (ii) LPC administered once i.p. at 25 mg/kg 1 h before bacterial inoculation, (iii) colistin administered i.p. at 20 mg/kg/8 h for 72 h, (iv) tigecycline administered s.c. at 5 mg/kg/12 h for 72 h, (v) imipenem administered i.m. at 30 mg/kg/4 h for 24 h, (vi) the combination of LPC with colistin, (vii) the combination of LPC with tigecycline, and (viii) the combination of LPC with imipenem. The antimicrobial dosages were chosen after obtaining the PK/PD data.

Mortality was recorded over 24 h (for imipenem treatment groups) or 72 h (for colistin and tigecycline treatment groups). After the death or euthanization of the mice at the end of the experimental period, aseptic thoracotomies were performed, and blood samples for qualitative blood cultures were obtained by cardiac puncture. Samples were inoculated into sterile tubes with 1 ml of LB and incubated for 24 h at 37°C, and then 100 μl was plated onto sheep blood agar. The results of the blood cultures are expressed as positive (when ≥1 CFU was present on the plate) or negative.

The spleen and lungs were aseptically removed and homogenized (Stomacher 80; Tekmar Co.) in 2 ml of sterile 0.9% NaCl solution. Tenfold dilutions of the homogenized spleen and lungs were plated onto sheep blood agar for quantitative cultures (to determine the log<sub>10</sub> CFU per gram of spleen or lung). If no growth was observed after plating the whole residue of the homogenized tissue, a logarithm value corresponding to the limit of detection of the method (1 CFU) was assigned. The limit of detection could then be determined as follows: CFU/g = 1 CFU/homogenized volume (ml) + weight (g).

Experimental murine model of pneumonia. A previously described experimental murine pneumonia model (8) was used to evaluate the efficacy of LPC as monotherapy and in combination with antimicrobial agents against the Ab186 strain. Briefly, the mice were anesthetized by an i.p. injection of 5% (wt/vol) sodium thiopental (Braun Medical, Barcelona, Spain). The mice were suspended vertically, and the trachea of each was then cannulated with a blunt-tipped metal needle. The feel of the needle tip against the tracheal cartilage confirmed the intratracheal location.

A microliter syringe (Hamilton Co., Reno, NV) was used for inoculation of 50 μl of a bacterial suspension (≈9 log CFU/ml) which had been grown for 24 h in LB broth at 37°C and mixed at a 1:1 ratio with a 0.9% NaCl solution containing 10% (wt/vol) porcine mucin. The mice remained in a vertical position for 3 min and then in a 30° position until they awakened. Treatment groups were similar to those for the experimental model of peritonial sepsis. After death or sacrifice of the mice at the end of the experimental period, aseptic thoracotomies were performed, and blood samples for qualitative blood culture were obtained by cardiac puncture (data are reported as numbers [%] of positive cultures). The lungs were aseptically removed and homogenized as described above for quantitative culture (data are reported in log<sub>10</sub> CFU per gram of lung).

**Cytokine assay.** Blood samples were collected from the peribotrial plexuses of 30 anesthetized mice infected with the Ab9 and Ab186 strains at the MLD<sub>100</sub>, as previously described (14). Serum levels of tumor necrosis factor alpha (TNF-α) and interleukin-10 (IL-10) were determined for mice at 0, 2, 4, 8, and 12 h postinfection by using an enzyme-linked immunosorbent assay (ELISA) (eBioscience).
RESULTS

Antimicrobial susceptibilities. The MICs of colistin, tigecycline, imipenem, and LPC for the Ab9 strain were ≤0.5, ≤0.25, ≤0.5, and 8,000 mg/liter, respectively. The MICs of colistin, tigecycline, imipenem, and LPC for the Ab186 strain were ≤0.5, 4, 16, and >8,000 mg/liter, respectively.

Bacterial effects on cytokine production. The effects of A. baumannii Ab9 and Ab186 on the serum TNF-α and IL-10 levels were examined from 0 to 12 h after induction of murine peritoneal sepsis (Fig. 1). The Ab9 strain induced more TNF-α release than the Ab186 strain from 0 to 8 h (Fig. 1, top panel), whereas the release of IL-10 at 4 h was significantly smaller with strain Ab9 than with strain Ab186 (P < 0.05) (Fig. 1, bottom panel).

Pharmacokinetic and pharmacodynamic parameters. The pharmacokinetic and pharmacodynamic data for total and free colistin, total tigecycline, and total imipenem are shown in Table 1.

MLD100, LD50, and LD0 of A. baumannii. To determine the MLD100, LD50, and LD0 of strains Ab9 and Ab186, the murine peritoneal sepsis model was used. Mortality was dependent on the concentration of bacteria in the inoculum (data not shown). The MLD100, LD50, and LD0 of strain Ab9 were 5.9, 4.05, and 2.3 log10 CFU/ml, respectively, and the MLD100, LD50, and LD0 of strain Ab186 were 5.3, 2.4, and 1.7 log10 CFU/ml, respectively. The LD50 of ATCC 17978 is 2.85 log10 CFU/ml (26).

Efficacies of LPC combination treatments in murine experimental model of peritoneal sepsis. The efficacies of LPC in combination with antimicrobial treatments, expressed as survival, bacterial concentrations in spleens and lungs, and percentages of sterile blood cultures, are shown in Tables 2 to 4.

(i) Survival. Tables 2 and 3, as well as Fig. S1 in the supplemental material, show that all treatments, alone or in combination, increased mouse survival compared with that of the control group for strains ATCC 17978 and Ab9 (P < 0.05). Pretreatment with LPC plus treatment with colistin or tigecycline increased mouse survival with strain ATCC 17978 compared to that obtained with pretreatment with LPC (P < 0.05) (Table 2). Pretreatment with LPC or treatment with LPC plus colistin, tigecycline, or imipenem increased mouse survival with strain Ab186 compared to that of the control group (P < 0.05).

(ii) Bacterial clearance from the spleen and lungs. Tables 2 to 4 show that LPC in combination with colistin decreased spleen

TABLE 1 Pharmacokinetic and pharmacodynamic parameters for single doses of colistin methanesulfonate, tigecycline, and imipenem

<table>
<thead>
<tr>
<th>Antimicrobial (dose [mg/kg], route of administration)</th>
<th>Drug form</th>
<th>Cmax (mg/liter)</th>
<th>$t_{1/2}$ (h)</th>
<th>AUC0–24 (mg · h/liter)</th>
<th>AUC0–24/MIC</th>
<th>$T_{MIC}$ (h, %)</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMS (20, i.p.)</td>
<td>CMS</td>
<td>14.24</td>
<td>1.12</td>
<td>43.24</td>
<td>172.97</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>tCST</td>
<td>2.87</td>
<td>1.1</td>
<td>14.41</td>
<td>57.63</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>fCST</td>
<td>0.97</td>
<td>1.01</td>
<td>4.7</td>
<td>18.81</td>
<td>ND</td>
</tr>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>TGC (5, s.c.)</td>
<td>fTGC</td>
<td>1.34</td>
<td>2.04</td>
<td>13.75</td>
<td>55</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>fIMP</td>
<td>26.66</td>
<td>0.36</td>
<td>ND</td>
<td>ND</td>
<td>1.54, 38.5</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMP (30, i.m.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* CMS, colistin methanesulfonate; TGC, tigecycline; IMP, imipenem; CST, colistin; fCST, total colistin; fTGC, total tigecycline; fIMP, total imipenem; Cmax, maximum concentration of antimicrobial agent in serum; $t_{1/2}$, elimination half-life; AUC0–24, area under the concentration-time curve from time zero to 24 h; $T_{MIC}$, time that the drug concentration remains above the MIC; ND, not determined; i.p., intraperitoneal; s.c., subcutaneous; i.m., intramuscular.

* For Ab9, the CST, TGC, and IMP MICs were 0.25, 0.25, and 0.5 mg/liter, respectively. For Ab186, the CST, TGC, and IMP MICs were 0.25, 4, and 16 mg/liter, respectively.
The Therapeutic Effect of LPC in Combination Therapy

Therapeutic effect of LPC in combination with colistin, tigecycline, or imipenem in murine peritoneal sepsis model with A. baumannii ATCC 17978

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Log10 CFU/g (mean ± SEM)</th>
<th>% sterile blood cultures</th>
<th>Mouse survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL</td>
<td>13</td>
<td>9.72 ± 0.09</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>LPC</td>
<td>15</td>
<td>5.81 ± 0.72</td>
<td>6.37 ± 0.62</td>
<td>40.00 ± 0.00</td>
</tr>
<tr>
<td>CST</td>
<td>15</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>LPC+CST</td>
<td>15</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>TGC</td>
<td>13</td>
<td>3.00 ± 0.57</td>
<td>4.60 ± 0.60</td>
<td>93.33 ± 0.00</td>
</tr>
<tr>
<td>TGC+TGC</td>
<td>15</td>
<td>0.35 ± 0.35</td>
<td>2.66 ± 0.58</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>IMP</td>
<td>15</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>IMP+IMP</td>
<td>15</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>100.00 ± 0.00</td>
</tr>
</tbody>
</table>

a CTL, control (no treatment); LPC, lysophosphatidylcholine; CST, colistin; TGC, tigecycline; IMP, imipenem.

b, * mouse mortality was recorded over 24 h; **, mouse mortality was recorded over 72 h.

c, P < 0.05 compared to the controls.
d, P < 0.05 compared to the LPC group.
e, P < 0.05 compared to the TGC group.

and lung concentrations of ATCC 17978, Ab9, and Ab186 by 9.72 and 8.74 log10 CFU/g (P < 0.05; ATCC 17978), respectively, 9.4 and 6.91 log10 CFU/g (P < 0.05; Ab9), respectively, and 6.08 and 5.73 log10 CFU/g (P < 0.05; Ab186), respectively, compared with the levels for the control groups. In contrast, monotherapy with colistin cleared ATCC 17978, Ab9, and Ab186 from the spleen and lungs by 9.72 and 8.74 log10 CFU/g (P < 0.05; ATCC 17978), respectively, 6.14 and 6.23 log10 CFU/g (P < 0.05; Ab9), respectively, and 4.31 and 3.18 log10 CFU/g (P < 0.05; Ab186), respectively, compared with the levels for the control groups. LPC in combination with colistin decreased spleen and lung concentrations of ATCC 17978, Ab9, and Ab186 by 5.81 and 6.37 log10 CFU/g (P < 0.05; ATCC 17978), respectively, 6.87 and 4.08 log10 CFU/g (P < 0.05; Ab9), respectively, and 4.02 and 3.55 log10 CFU/g (P < 0.05; Ab186), respectively, compared with the levels for the LPC pretreatment group.

LPC in combination with tigecycline decreased spleen and lung concentrations of ATCC 17978, Ab9, and Ab186 by 9.37 and 6.08 log10 CFU/g (P < 0.05; ATCC 17978), respectively.

9.01 log10 CFU/g (P < 0.05; Ab9), respectively, and 2.98 and 3.16 log10 CFU/g (P < 0.05; Ab186), respectively, compared with the levels for the control groups. In contrast, tigecycline reduced the spleen and lung concentrations of ATCC 17978, Ab9, and Ab186 by 6.72 and 4.14 log10 CFU/g (P < 0.05; ATCC 17978), respectively, 8.57 and 8.66 log10 CFU/g (P < 0.05; Ab9), respectively, and 0.19 and 0.04 log10 CFU/g (Ab186), respectively, compared with the levels for the control groups. LPC in combination with tigecycline decreased spleen and lung concentrations of ATCC 17978, Ab9, and Ab186 by 5.46 and 3.71 log10 CFU/g (P < 0.05; ATCC 17978), respectively, 6.7 and 6.18 log10 CFU/g (P < 0.05; Ab9), respectively, and 0.92 and 0.98 log10 CFU/g (Ab186), respectively, compared with the levels for the LPC pretreatment group.

Finally, LPC in combination with imipenem decreased spleen and lung concentrations of ATCC 17978, Ab9, and Ab186 by 9.72 and 8.74 log10 CFU/g (P < 0.05; ATCC 17978), respectively, 8.41 and 8.74 log10 CFU/g (P < 0.05; Ab9), respectively, and 3.18 and 2.27 log10 CFU/g (P < 0.05; Ab186), respectively, compared with the levels for the control groups. In contrast, imipenem reduced spleen and lung concentrations of ATCC 17978, Ab9, and Ab186 by 9.72 and 8.74 log10 CFU/g (P < 0.05; ATCC 17978), respectively, 5.88 and 5.91 log10 CFU/g (P < 0.05; Ab9), respectively, and 1.12 and 0.09 log10 CFU/g (Ab186), respectively, compared with the levels for the LPC pretreatment group.

Interestingly, the calculation of mean values for bacterial burdens of dead and live mice separately within the same treatment group for the ATCC 17978, Ab9, and Ab186 strains showed differences (see Tables S1 to S3 in the supplemental material). For the group receiving LPC in combination with colistin against strain Ab186, dead mice (n = 5) had higher bacterial burdens (9.3 ± 0.31 log CFU/g in the spleen and 9.66 ± 0.14 log CFU/g in the lungs) than those of live mice (n = 10) (0.92 ± 0.38 log10 CFU/g in the spleen and 1.02 ± 0.43 log10 CFU/g in the lungs) (see Table S3). Similarly, for the group receiving LPC in combination with...
TABLE 5 Therapeutic effect of LPC in combination with colistin, tigecycline, or imipenem in murine pneumonia model with A. baumannii Ab186

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Log₁₀ CFU/g lung (mean ± SEM)</th>
<th>% sterile blood cultures</th>
<th>Mouse survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL</td>
<td>8</td>
<td>10.93 ± 0.17</td>
<td>0</td>
<td>0*</td>
</tr>
<tr>
<td>LPC</td>
<td>8</td>
<td>6.12 ± 0.98</td>
<td>50*</td>
<td>50**</td>
</tr>
<tr>
<td>CST</td>
<td>15</td>
<td>5.74 ± 1.05</td>
<td>26.67d</td>
<td>60**</td>
</tr>
<tr>
<td>LPC+CST</td>
<td>15</td>
<td>3.91 ± 0.88</td>
<td>73.33e</td>
<td>73.33***</td>
</tr>
<tr>
<td>TGC</td>
<td>14</td>
<td>8.00 ± 0.94</td>
<td>50*</td>
<td>50**</td>
</tr>
<tr>
<td>LPC+TGC</td>
<td>15</td>
<td>5.11 ± 0.94</td>
<td>66.67d</td>
<td>66.67***</td>
</tr>
<tr>
<td>IMP</td>
<td>12</td>
<td>6.85 ± 0.44</td>
<td>25c</td>
<td>100**</td>
</tr>
<tr>
<td>LPC+IMP</td>
<td>15</td>
<td>4.42 ± 0.75e</td>
<td>26.67d</td>
<td>100**</td>
</tr>
</tbody>
</table>

* CTL, control (no treatment); LPC, lysophosphatidylcholine; CST, colistin; TGC, tigecycline; IMP, imipenem.

a,b,c,d,e P < 0.05 compared to the CST, TGC, or IMP group.

(ii) Bacterial clearance from lungs. For ATCC 17978, LPC in combination with colistin reduced the lung concentration of the Ab186 strain by 6.51 and 4.08 log₁₀ CFU/g (P < 0.05), respectively, compared with the level for the control group. LPC in combination with imipenem monotherapy decreased the lung concentration of the Ab186 strain by 5.82 log₁₀ CFU/g (P < 0.05) and 2.93 log₁₀ CFU/g, respectively, compared with the level for the control group. Finally, LPC in combination with colistin, tigecycline, or imipenem decreased the lung concentration of strain Ab186 by 2.26, 1.06, and 1.75 log₁₀ CFU/g (P < 0.05), respectively, compared with the level for the LPC pretreatment group.

Interestingly, the calculation of mean values for bacterial burdens of dead and live mice separately within the same treatment group for strain Ab186 showed results similar to those for the murine peritoneal sepsis model with Ab186 (see Table S4 in the supplemental material).

(iii) Bacterial clearance from blood. LPC in combination with colistin increased the percentage of sterile blood cultures compared with those for colistin and LPC monotherapies and the control group (P < 0.05). LPC in combination with tigecycline presented a trend toward fewer sterile blood cultures than those with tigecycline and LPC monotherapies (33.33% versus 50% and 50%, respectively). In contrast, LPC in combination with imipenem did not increase the sterility of blood cultures compared to that with imipenem monotherapy (Table 5).

DISCUSSION

Previous studies from our group demonstrated that preemptive LPC therapy protected mice from A. baumannii infections, reducing bacterial organ loads and bacteremia and increasing mouse survival to 40% (14), similar to the results obtained with both strains used in the present study. In addition, we showed that surviving mice had lower bacterial burdens in tissues than those in dead mice, which explains the observed improvement in survival. Based on this positive therapeutic effect of LPC, we hypothesized that it may be an adjuvant to antimicrobial therapy for patients at risk of severe A. baumannii infection (14). Thus, murine experimental models of peritoneal sepsis and pneumonia were performed to evaluate the efficacy of LPC in combination with colistin, tigecycline, or imipenem.

Nowadays, the high prevalence of MDR A. baumannii reduces treatment options. Colistin and tigecycline are among the last treatments available worldwide, due to their low resistance rates (27, 28). Imipenem remains the gold standard treatment for infections with susceptible strains, and it is widely used in the clinical setting (29). In the present study, monotherapy with colistin against susceptible and MDR A. baumannii strains significantly reduced bacterial spleen and lung concentrations and bacteremia and increased mouse survival.

Interestingly, in the experimental model of peritoneal sepsis, pretreatment with LPC in combination with colistin treatment reduced the bacterial loads in tissues and the proportion of bacteremia compared to those for colistin treatment alone. Accordingly, the LPC-colistin combination also increased mouse survival. Both A. baumannii strains are susceptible to colistin; however, the spleen and lung bacterial loads were 2 log higher for the MDR strain than for the susceptible strain after treatment with
colistin or pretreatment with LPC plus treatment with colistin. This difference in bacterial load was not due to different pharmacodynamics between the strains, because the MIC of Ab9 and Ab186 was 0.5 μg/ml, which is equal to the MIC for both strains before inoculation into mice. We suggest that the difference in bacterial loads may be due to the difference in immune responses caused by both strains. Indeed, Fig. 1 shows that the susceptible strain, the high efficacy of imipenem or tigecycline against A. baumannii, possibly due at least to the alteration in vivo of proinflammatory cytokines (TNF-α, IL-17, gamma interferon [IFN-γ], and IL-1β) (32–34). In an in vivo study, Qiu et al. associated the susceptibility of mice to A. baumannii infection with local proinflammatory cytokine responses, including TNF-α responses, and with a delay in the early influx of neutrophils into the lung (35). In contrast, infection of lipopolysaccharide (LPS) by an LpxC inhibitor suppressed A. baumannii LPS-mediated activation of TLR4 and consequently reduced inflammation in vivo (36).

With respect to the usefulness of the combination of LPC with tigecycline or imipenem, we observed a trend toward a decrease in bacterial tissue concentrations compared to those with antimicrobial monotherapies, but without statistical significance. In the case of the experimental model of peritoneal sepsis caused by the susceptible Ab9 strain, the high efficacy of imipenem or tigecycline alone (bacterial concentrations in tissues were 1 to 1.5 log₁₀ CFU/g) precluded the observation of a larger reduction with the combined treatment. In the case of the experimental models of peritoneal sepsis and pneumonia caused by the MDR strain, as expected, no therapeutic effect was observed with tigecycline or imipenem treatment due to resistance to both antimicrobials. However, in mice treated with LPC in combination with tigecycline or imipenem, bacterial loads were significantly reduced compared to those for the controls and the tigecycline- or imipenem-treated group. Although LPC plus tigecycline or imipenem reduced the bacterial concentrations in tissues (≈1 to 2 log₁₀ CFU/g) compared to those with LPC monotherapy, these differences were not significant, as the results were not different in terms of bacteremia and survival. These data show that LPC has a moderate impact when there is no antibiotic efficacy because of high drug resistance.

The LPC prophylactic treatment model has some limitations regarding the animal model system and the LPC treatment regimen. We believe that the next issues to be addressed are as follows: (i) to determine the bacterial burdens in tissues and bacteria only for live and moribund mice to avoid any effect of death for both control and treated groups in order to confirm the therapeutic efficacy of LPC in combination with antimicrobial agents observed in the present study; (ii) to determine whether multiple doses of LPC given as a treatment in combination with antimicrobial agents can improve the effect of LPC; and (iii) to use BALB/c mice as another animal model, since BALB/c mice are Th2 biased, while C57BL/C6 mice are Th1 biased (37).

It is important that other immunomodulation applications have been performed, such as application of granulocyte colony-stimulating factor (G-CSF) as an adjuvant with antibiotics and application of corticosteroids as an adjunct treatment for pneumonia (38, 39). Positive results have been seen in animals for both applications, but when mixed with clinical results, G-CSF application does not mimic the results seen with the animal model (38).

In summary, the present study suggests that LPC pretreatment in combination with colistin, tigecycline, or imipenem treatment improves the in vivo antibacterial activity in cases of experimental peritoneal sepsis and pneumonia caused by A. baumannii.

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