Efficacy and pharmacokinetics of ME1100, a novel optimized formulation of arbekacin for inhalation, compared to amikacin in a murine model of ventilator-associated pneumonia caused by *Pseudomonas aeruginosa*

Running Title:

Efficacy and pharmacokinetics of ME1100 in VAP

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Abstract:

Background: Arbekacin is an aminoglycoside that shows a strong antimicrobial activity against Gram-positive bacteria, including MRSA as well as *Pseudomonas aeruginosa*. The therapeutic effectiveness of arbekacin is directly related to the mean maximum drug concentration (C_{max}) at the infection site. To maximize drug delivery to the respiratory tract and minimize the systemic toxicity, arbekacin optimized for inhalation, ME1100, is under development. In this study, we investigated the efficacy and pharmacokinetics of ME1100 in a murine model of ventilator-associated pneumonia caused by *P. aeruginosa* by using customized investigational nebulizer system.

Methods: The mice were treated for 5 minutes, once daily, with placebo, 3, 10, or 30 mg/mL of ME1100 or 30 mg/mL of amikacin.

Results: In the survival study, the survival rate was significantly improved in the 10 and 30 mg/mL ME1100 treatment groups, compared with that in the placebo group. The number of bacteria in the lungs significantly decreased in the 30 mg/mL ME1100 treatment group at 6 hours after the initial treatment. In the pharmacokinetic study, the C_{max} of the 30 mg/mL ME1100 treatment group in the epithelial lining fluid (ELF) and plasma was 31.1 μg/mL and 1.2 μg/mL, respectively. Furthermore, we compared the efficacy of ME1100 with that of amikacin. Although there were no significant differences in the ELF and plasma concentrations between ME1100 and amikacin, ME1100 significantly improved the survival rate, compared to amikacin.

Conclusions: The results of our study demonstrated the *in vivo* effectiveness of ME1100 and its superiority to amikacin.

Introduction:

Arbekacin is an aminoglycoside, which induces cell membrane damage and binds to both the 50S and 30S ribosomal subunits, resulting in codon misreading and inhibition of translation.[1] It shows a strong antimicrobial activity against Gram-positive bacteria, including MRSA. The antimicrobial activity of arbekacin against MRSA was equivalent to that of vancomycin.[1,2,3,4] Additionally, arbekacin shows a good antimicrobial activity against Gram-negative bacteria including *Pseudomonas aerunigosa*.[2,5] Since *S. aureus* and *P. aeruginosa* are the most causative pathogens of HAP including ventilator-associated pneumonia (VAP),[5,6] arbekacin might be useful for the treatment of patients with these diseases.

The therapeutic effectiveness of arbekacin is directly related to the fraction of the mean maximum drug concentration (C_{max}) at the infection site. The ratio of the C_{max} of arbekacin in the epithelial lining fluid (ELF) to that in the serum is 0.40.[7] This data suggested that a higher dose is needed to treat patients with pneumonia than that needed for those with bloodstream infections. However, a higher dose will lead to a higher trough concentration (C_{trough}). It is reported that a high C_{trough} of aminoglycosides increases the incidence of kidney-related adverse drug reactions, and therapeutic drug monitoring (TDM) is required to maximize the efficacy while minimizing the toxicity of aminoglycosides.[1] In the 1990s, inhalation aminoglycosides, such as tobramycin and amikacin, were developed to maximize the drug delivery to the respiratory tract and minimize the toxicity. In this study, we investigated the *in vivo* efficacy of ME1100, a novel optimized formulation of arbekacin for inhalation, by using a customized investigational nebulizer system, and compared its efficacy with that of amikacin.

Materials and methods:

Bacterial strains:

The strain of *Pseudomonas aeruginosa* used in this study was PANU3724, a clinical isolate obtained from the endotracheal tube of a patient at the Nagasaki University Hospital. The bacteria were stored at -80 °C in a Microbank® bead preservation system (Pro-Lab Diagnostics, Ontario, CA) until use.

Antimicrobial agents:

Amikacin sulphate injection was purchased from Sawai Pharmaceutical Co., Ltd. (Osaka, Japan). ME1100 inhalation solution and placebo inhalation solution were supplied by Meiji Seika Pharma Co., Ltd. (Tokyo, Japan). ME1100 is a novel formulation of arbekacin, which provides high concentration and stability for inhalation (International Application No. PCT/EP2012/065265). The aqueous liquid pharmaceutical composition of ME1100 inhalation solution is comprised by arbekacin and chloride ions. By the composition, the sulfate salt form of the arbekacin, which caused coughing reaction, was reduced.

Inhalation exposure system:

The customized investigational eFlow® rapid nebulizer system was supplied by Pari Pharma GmbH. The average of the Mass Median Aerodynamic Diameter of the ME1100 droplets generated by the nebulizer system was 3.7 ± 0.1 μm, and approximately 73% of the droplets were in the size range of 0.5 to 5 μm.[8] In this study, we constructed the original nose-only exposure system. We cut the tip of the 50 mL conical centrifuge tubes, and held the mice in the tube with a posture to put out their nose from the tip of the tubes.

Subsequently, we put the tubes into a sealed container which the flow generated by the nebulizer system passed through.

Animals:

We purchased specific pathogen-free male ICR mice (6 to 7-week-old, 25 to 30 g bodyweight) from Japan SLC, Inc. (Shizuoka, Japan). The mice were housed in a pathogen-free environment and received sterile food and water in the Biomedical Research Center at Nagasaki University.

Ethics:

All the experimental protocols used in this study were approved by the Ethics Review Committee for Animal Experimentation (approval number: 1003310842).

Antimicrobial susceptibility test:

We determined the MIC of the antimicrobial agents against the PANU3724 strain by a micro-dilution method in accordance with the guidelines of the CLSI.[9] CLSI interpretative criteria (M100-S24) were applied for testing the susceptibility of the PANU3724 strain to the antimicrobial agents.[10]

Murine model of VAP:

Disposable sterile plastic cut-down intravenous catheters with a French gauge of 3-Fr (Atom Co., Tokyo, Japan) were used for tracheal intubation. The catheters were cut to a length of 5.0 mm and a few slits were made at the proximal end to prevent their clogging by oral secretions. The bacteria were cultured overnight on a Muller-Hinton II agar (Becton Dickinson, Le Pont de Claix, France). Then, they were suspended in sterile saline

and the concentration was adjusted at 2 x 10^8 cfu/mL, as estimated by turbidimetry (DensiCHEK™ plus: bioMérieux, Hazelwood, MO). Inoculation was carried out as described previously.[11,12,13,14] Briefly, the tubes were inserted through the vocal cords into the trachea. Subsequently, *P. aeruginosa* suspended in saline solution (0.05 mL; 1 x 10^7 cfu/mouse) was inoculated through the outer sheath of the intravenous catheter.

Treatment protocol:

ME1100 and amikacin were diluted with the placebo inhalation solution. Twelve hours post inoculation, the mice were treated for 5 minutes, once daily, with placebo, 3, 10, or 30 mg/mL of ME1100, or 30 mg/mL of amikacin by using a customized investigational nebulizer system. In the survival study, the mice were treated for 108 hours post inoculation and the survival rates were observed for 120 hours post inoculation.

Bacteriological examinations:

The mice were sacrificed at 18 hours post inoculation i.e., 6 hours after the initial treatment, by cervical dislocation. Subsequently, they were dissected under aseptic conditions to collect the blood *via* a right ventricular puncture using heparin-coated syringes, and to remove the lungs. The lungs were suspended in 1 mL of normal saline and homogenized with a homogenizer (AS One Co., Osaka, Japan). Serial dilutions of the lungs and blood were quantitatively cultured in Mueller-Hinton II agar plates. After overnight incubation, we evaluated the number of the visible colonies. The lowest level of detectable bacterial count was 1 x 10^2 cfu/mL.

Collection of blood and bronchoalveolar lavage fluid (BALF) for the pharmacokinetic studies:

The mice were treated for 5 minutes with 30 mg/mL of ME1100 or 30 mg/mL of amikacin by using a customized investigational nebulizer system, and were then sacrificed by cervical dislocation at 5, 30 min, 1, 3, 6, and 12 hours post inhalation. Blood was collected *via* a right ventricular puncture using heparin-coated syringes. The bronchoalveolar lavage fluid (BALF) was also collected. Four mice were used for each group. The collection of BALF was performed as described previously.[15,12] Briefly, the mice were sacrificed and the chest opened to expose the lungs and trachea. A disposable, sterile, plastic cut-down catheter was inserted into the trachea. BAL was performed three times sequentially using 1.0 mL of saline each time. Blood and BALF were centrifuged and the supernatants were stored at -80 °C until use.

Pharmacokinetic studies:

The levels of arbekacin and amikacin in the plasma and BALF were measured using a LC (LC-10Advp, Shimadzu) coupled with a tandem mass spectrometer (Quattro Ultima Pt, waters). Dibekacin was used as an internal standard (I.S.). All analytes were determined *via* measurement of a derivative formed by their reaction with phenyl isocyanate in the presence of triethylamine.

When the value of an assessed parameter of a PK sample was expected to exceed the upper limit of quantification (ULOQ), the sample was diluted 2-fold (plasma) or 5-fold (BALF) with blank plasma or BALF. Each plasma or BALF sample (10 μL) was transferred to a tube, and the standard solution (10 μL) and saline (180 μL) were added and mixed with a vortex mixer. The mixture (50 μL) was transferred to another tube, and the internal standard solution (50 μL), acetonitrile containing 0.5 % triethylamine (50 μL), and acetonitrile containing 0.5 % phenyl isocyanate (50 μL) were added and

169 mixed with the vortex mixer. Then, methanol (400 μL) was added. The mixture was
170 centrifuged at 9100 $\times g$ for 5 min at 4 °C. The supernatant (30 μL) was injected into
171 the column of the liquid chromatograph-tandem mass spectrometry system. Arbekacin
172 and amikacin were chromatographically separated on an analytical column (Inertsil ODS-
173 3, 2.1x50 mm, 5 μm, GL Sciences Inc.) using a gradient of water/formic acid (1000:1,
174 v/v) – acetonitrile as a mobile phase at 40 °C. The flow rate was set at 0.4 mL/min. The
175 tandem mass spectrometer was operated in the positive ion mode. Arbekacin was
176 monitored as the precursor ion at 1149 m/z and the product ion at 367 m/z. Amikacin was
177 monitored as the precursor ion at 1062 m/z and the product ion at 409 m/z. Dibekacin
178 (I.S.) was monitored as the precursor ion at 1048 m/z and the product ion at 367 m/z. The
179 assay was linear over 0.05 to 5 mg/L for arbekacin and amikacin in the mouse plasma
180 and BALF. The accuracy of the assay method ranged between 88.9 % and 114.2 %.

181 Measurement of urea concentration in the plasma and BALF was conducted using urea
182 assay kit (Biochain Institute, Inc) based on Jung method.[16] The samples were diluted 50-
183 fold (plasma) or 10-fold (BALF) with saline. The standard curve showed a linear range
184 from 0.05 to 1 mg/dL. Arbekacin and amikacin concentrations in the ELF were calculated
185 using the following equation because the ELF was diluted with saline: Arbekacin (or
186 Amikacin) in ELF = Arbekacin (or Amikacin) in BALF × Urea in plasma / Urea in
187 BALF.[17]

188 For pharmacokinetic analysis, WinNonlin Professional Ver.6.3 (Pharsight Corporation)
189 was used.

190

191 *Statistical analysis:*

We used a statistical software package (StatMate V; ATMS Co., Ltd., Tokyo, Japan) for all the statistical comparisons. The survival rates were calculated using Kaplan-Meier method. The survival analysis was performed using the log-rank test and the data was expressed as the mean ± standard deviation (SD). For the plot of the bacterial count in the lungs and blood, we depicted the data using the box-and-whisker plot and analysed the differences between the groups using one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. All the tests of significance were two-tailed. The alpha level for denoting a statistical significance was set at $p < 0.05$.

Results:

MICs of the antimicrobial agents against PANU3724:

The MICs of arbekacin and amikacin against the bacterial strain, PANU3724, were 4 mg/L and 8 mg/L, respectively. This bacterial strain exhibits resistance to carbapenems (Table 1).

Therapeutic effects of the antimicrobial agents on the survival rate:

In the survival study, the mice were treated according to the prescribed methods for 108 hours post inoculation and the survival rates were observed for 120 hours post inoculation ($n = 7$ in each group). As shown in Fig. 1, the survival rates were significantly higher in the 10 and 30 mg/mL ME1100 treatment groups than in the placebo treatment group ($P < 0.001$ for both groups). Additionally, the survival rate in the 30 mg/mL ME1100 treatment group was significantly higher than that in the placebo treatment group ($P = 0.022$).

Bacteriological examinations:

Since some mice in the placebo treatment group died at 24 hours post inoculation in the survival study, the mice were sacrificed 18 hours post inoculation i.e., 6 hours after the initial treatment ($n = 5$ in each group) .

The bacterial count in the lungs of the mice receiving placebo, 3, 10, and 30 mg/mL of ME1100 was as follows: 9.34 ± 0.23, 9.20 ± 0.33, 8.86 ± 0.31, and 8.09 ± 0.35 $\log_{10}$ cfu/mL, respectively (Fig. 2A). The bacterial count in the lungs significantly decreased in the 30 mg/mL ME1100 treatment group, compared to that in all other groups ($P < 0.001$ *versus* the placebo and the 3 mg/mLME1100 treatment groups; $P < 0.05$ *versus* the 10 mg/mL ME1100 treatment group).

The bacterial count in the blood of the mice receiving placebo, 3, 10, and 30 mg/mL of ME1100 was as follows: 6.08 ± 0.43, 5.36 ± 0.96, 4.91 ± 0.64, and 4.39 ± 1.47 $\log_{10}$ cfu/mL, respectively (Fig. 2B). The bacterial count in the blood significantly decreased in the 30 mg/mL ME1100 treatment group, compared to that in all other groups ($P < 0.001$ *versus* the placebo and the 3 mg/mLME1100 treatment groups; $P < 0.05$ *versus the* 10 mg/mL ME1100 treatment group).

Comparison of ME1100 and amikacin:

In the survival study, the mice were treated according to the prescribed methods for 108 hours post inoculation and the survival rates were observed for 120 hours post inoculation ($n = 6$ in each group). As shown in Fig. 3A, the survival rates were significantly higher in the 30 mg/mL ME1100 treatment group than in the placebo and 30 mg/mL amikacin treatment groups ($P < 0.005$ *versus* placebo and $P = 0.024$ *versus* amikacin).

In the bacteriological examination, the mice were sacrificed 18 hours post inoculation i.e., 6 hours after the initial treatment ($n = 5$ in each group). The bacterial counts in the lungs of the mice in the placebo, 30 mg/mL ME1100, and 30 mg/mL amikacin treatment groups were 10.23 ± 0.24, 9.51 ± 0.27, and 10.20 ± 0.05 $\log_{10}$ cfu/mL, respectively (Fig. 3B). The bacterial count in the lungs significantly decreased in the 30 mg/mL ME1100 treatment group, compared to that in the placebo and amikacin treatment groups ($P < 0.001$ *versus* both groups).

Pharmacokinetics of ME1100

The mice were treated for 5 minutes with 30 mg/mL of ME1100, and then, ELF and blood were collected at 5, 30 minutes, 1, 3, 6, and 12 hours post inhalation. The calculated pharmacokinetic parameters of ME1100 are shown in Fig. 4A and Table 2. The C_{max} of

ME1100 in the ELF and plasma was 31.1 and 1.2 μg/mL, respectively. The areas under the concentration-time curve from 0 to infinity ($AUC_{0\text{-}inf}$) of ME1100 in the ELF and plasma were 67.7 and 2.2 μg.h/mL, respectively. The half-lives ($t_{1/2}$) of ME1100 in the ELF and plasma were 2.4 and 4.6 h, respectively. The ELF concentration of ME1100 was significantly higher than its plasma concentration at 5, 30 minutes, 1 and 3 hours post inhalation ($P = 0.028$).

Comparison of the ELF and blood concentrations of ME1100 and amikacin

The mice were treated for 5 minutes with 30 mg/mL of ME1100 or amikacin, and then ELF and blood were collected at 5 minutes and 1 hour post inhalation. The concentration of ME1100 in the ELF at 5 minutes and 1 hour post inhalation was 31.1 ± 13.6 and 20.2 ± 17.6, respectively, and that of amikacin was 46.7 ± 16.1 and 19.1 ± 13.3, respectively (Fig. 4B). The concentration of ME1100 in the plasma at 5 minutes and 1 hour post inhalation was 1.2 ± 0.5 and 0.4 ± 0.1, respectively, and that of amikacin was 2.5 ± 1.7 and 0.5 ± 0.2, respectively (Fig. 4C). There was no significant difference between the ELF and blood concentrations of ME1100 and amikacin.

Discussion:

Antimicrobial agents, such as tobramycin, colistin, and aztreonam, have been administered *via* inhalation to patients with chronic respiratory tract infections caused by *P. aeruginosa*. *P. aeruginosa* has been a common cause of HAP including VAP.[5,6] Although there are several anti-pseudomonal agents, *P. aeruginosa* is associated with high in-hospital mortality and prolonged length of stay in hospitals.[18] The treatment of *P. aeruginosa* infection has been difficult because the bacteria possess numerous mechanisms of antimicrobial resistance. Therefore, there is a need to develop novel antimicrobial agents effective against *P. aeruginosa*. In the ATS / IDSA guidelines, combination therapy with aminoglycosides and beta-lactams was recommended as an empiric treatment for patients with VAP caused by drug-resistant Gram-negative bacteria including *P. aeruginosa.*[19] However, this combination therapy for sepsis was discouraged due to the significant risk of nephrotoxicity.[20] To decrease the risk of nephrotoxicity, some aminoglycosides formulations were optimized in order to be administered *via* inhalation in patients with VAP.[21] In this study, ME1100, optimized for inhalation, showed a good *in-vivo* antimicrobial activity in a murine model of VAP caused by *P. aeruginosa*. In the survival study, ME1100 treatment (30 mg/mL) significantly improved the survival rate in comparison with the placebo group, in which the infected mice died within 48 hours post inoculation. In addition, ME1100 (30 mg/mL) significantly reduced the bacterial count in the lungs and blood when administered only once.

The primary purpose of the inhalation route is to maximize the drug delivery to the target site of infection and limit the potential for systemic side effects. Arbekacin, a conventional formulation of ME1100, is classified as a kanamycin aminoglycoside. The therapeutic effectiveness of aminoglycosides is directly related to the C_{max}, and the previous study reported that the probability of cure/improvement was risen when the C_{max}

295 of arbekacin was increased.[22] In patients with pneumonia, the local concentration of the
296 antimicrobial agents at the infection site, such as ELF, is important.[23] A previous study on
297 the intravenous administration of arbekacin reported that the ratio of the C_{max} of arbekacin
298 in the ELF to that in the serum is 0.40.[7] These data suggested that a higher dose of
299 arbekacin would be needed to treat patients with pneumonia *via* intravenous
300 administration rather than *via* inhalation. In this study, the ratio of the C_{max} of ME1100 in
301 the ELF to that in the plasma was approximately 25.9. Accordingly, ME1100 could
302 contribute to the treatment of patients with VAP by maximizing the drug delivery to the
303 respiratory tract. However, there is a possibility of the resistance emergence in the patients
304 with bacteremia because the low C_{max} of ME1100 in the plasma would facilitate the drug-
305 resistance. Therefore, as described in the ATS / IDSA guidelines[19], the combination of
306 ME1100 and beta-lactams might be recommended in clinical use.
307 Among the aminoglycosides optimized for inhalation, amikacin is frequently reported.
308 Several clinical studies reported that administration of amikacin *via* inhalation is effective
309 in patients with VAP.[24,25,26] In this study, we compared the *in vivo* efficacy and
310 pharmacokinetics of ME1100 with that of amikacin. Although there were no significant
311 differences in the drug concentrations in the ELF and plasma between ME1100 and
312 amikacin, ME1100 significantly improved the survival rate, compared to amikacin.
313 However, the nebulizer system used in this study was customized for inhalation of
314 ME1100. Additionally, we did not adjust the doses of ME1100 and amikacin based on
315 their pharmacokinetics. The MIC of amikacin against PANU3724 strain is higher than
316 that of ME1100, and the calculated C_{max} / MIC of amikacin was lower than that of
317 ME1100 (5.8 for amikacin and 7.8 for ME1100, respectively). Since the optimized
318 formulation of amikacin for inhalation, known as BAY41-6551 or NKTR-061 is under
319 development[24], it is necessary to compare ME1100 and the optimized amikacin

formulation for inhalation in the future.

Some previous studies reported that arbekacin shows a strong antimicrobial activity against Gram-positive bacteria including MRSA.[3,4] Since arbekacin is stable to the aminoglycoside-inactivating enzymes produced by MRSA, it shows the most potent antimicrobial activity against MRSA among the aminoglycosides.[1] Additionally, the MIC required to inhibit the growth of 90% of organisms (MIC_{90}) of arbekacin against MRSA is 0.5 μg/mL, which is lower than that of vancomycin and linezolid (1 and 2 μg/mL, respectively).[1] Since *P. aeruginosa* and *S. aureus,* including MRSA are the most and the second most frequent isolated pathogens from patients with VAP,[5] ME1100 might be useful for the treatment of patients with VAP.

There were some limitations in this study. First, we used only one clinical strain of *P. aeruginosa* and one mouse species in this study. Second, it is very difficult to predict the efficacy of ME1100 in human because ME1100 is still under development and the pharmacokinetics of ME1100 in human remains unknown. Third, in this study, the amikacin sulphate injection used, was not optimized for inhalation. Finally, we did not investigate the toxicity of ME1100 and amikacin in this study.

In conclusion, the results of our study demonstrated the *in vivo* effectiveness of ME1100 and its superiority to amikacin. Further investigations, including clinical trials, are needed to determine the efficacy of ME1100 in patients with VAP.

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Transparency declarations:

None to declare

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435 **Tables:**

Table 1. The MICs of antimicrobial agents against the PANU3724

	PIPC/TAZ	CAZ	AZT	IPM	MEPM	DRPM	GM	AMK	ME1100	CPFX
MIC (g/L)	32	8	16	32	16	8	4	8	4	1
Susceptibility [a]	I	S	I	R	R	R	S	S	NA	S

The susceptibility of 10 antimicrobial agents against PANU3724 was determined. PIPC/TAZ, piperacillin – tazobactam (PIPC : TAZ = 8 : 1); CAZ, ceftadizime; AZT, aztreonam; IPM, imipenem; MEPM, meropenem; DRPM, doripenem; GM, gentamicin; AMK, amikacin; ME1100, arbekacin ihalation solution; CPFX, ciprofloxacin.

[a] Criteria as M100-S24 published by CLSI. S, susceptible; I, intermediate; R, resistant; NA, no criteria available.

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Table 2. Selected pharmacokinetic parameters estimated for ME1100 in ELF and plasma

	C_{max}	t_{max}	$t_{1/2}$	$AUC_{0\text{-}inf}$
	(μg/mL)	**(h)**	**(h)**	μg h/mL
ELF	31.1	0.08	2.4	67.8
Plasma	1.2	0.08	4.6	2.9

C_{max}, maximum drug concentration; t_{max}, time to maximum concentration; $t_{1/2}$, half-life; $AUC_{0\text{-}inf}$, area under the concentration-time curve from 0 to infinity.

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440 **Figures:**

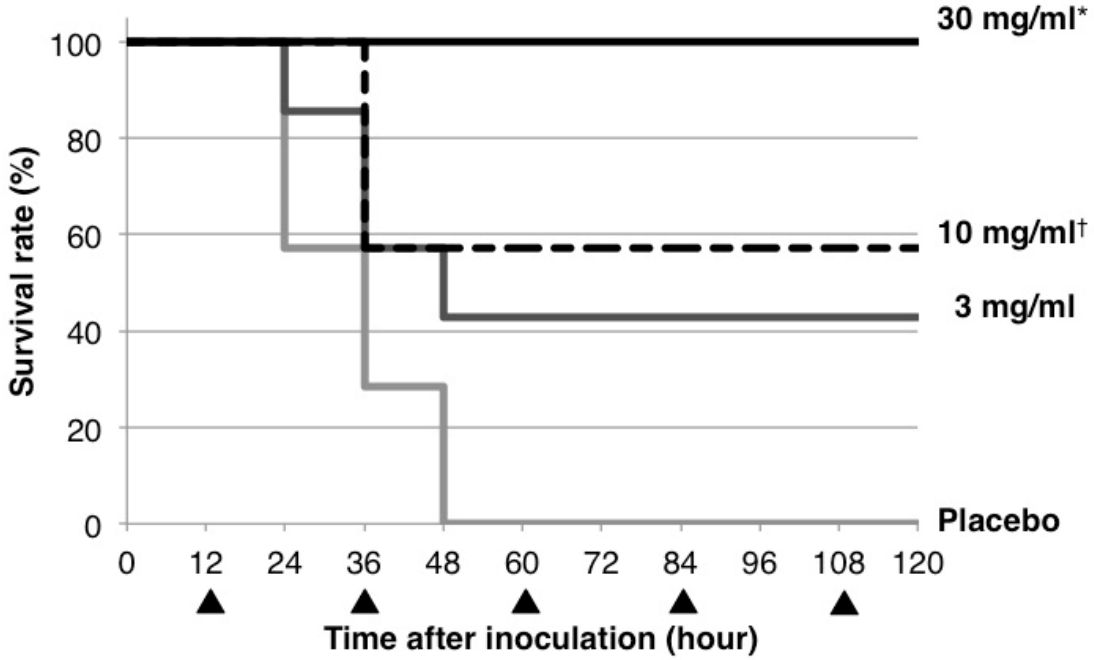

Fig. 1. Therapeutic effects of ME1100 on survival rate.
Twelve hours post inoculation, the mice were treated for 5 minutes, once daily, with placebo, 3, 10, or 30 mg/mL of ME1100. Survival was estimated at the indicated time, and the results are displayed as a Kaplan-Meier plot. The black triangles represent the time of treatment. The survival rates were observed for 120 hours after inoculation ($n = 7$ in each group). * The survival rates in the 30 mg/mL ME1100 treatment group was significantly higher than that in the placebo and 3 mg/mLME1100 treatment groups ($P < 0.001$ *versus* placebo, and $P = 0.022$ *versus* the 3 mg/ mL of ME1100).† The survival rates in the 10 mg/ mL ME1100 treatment group were significantly higher than those in the placebo group ($P <$ 0.001).

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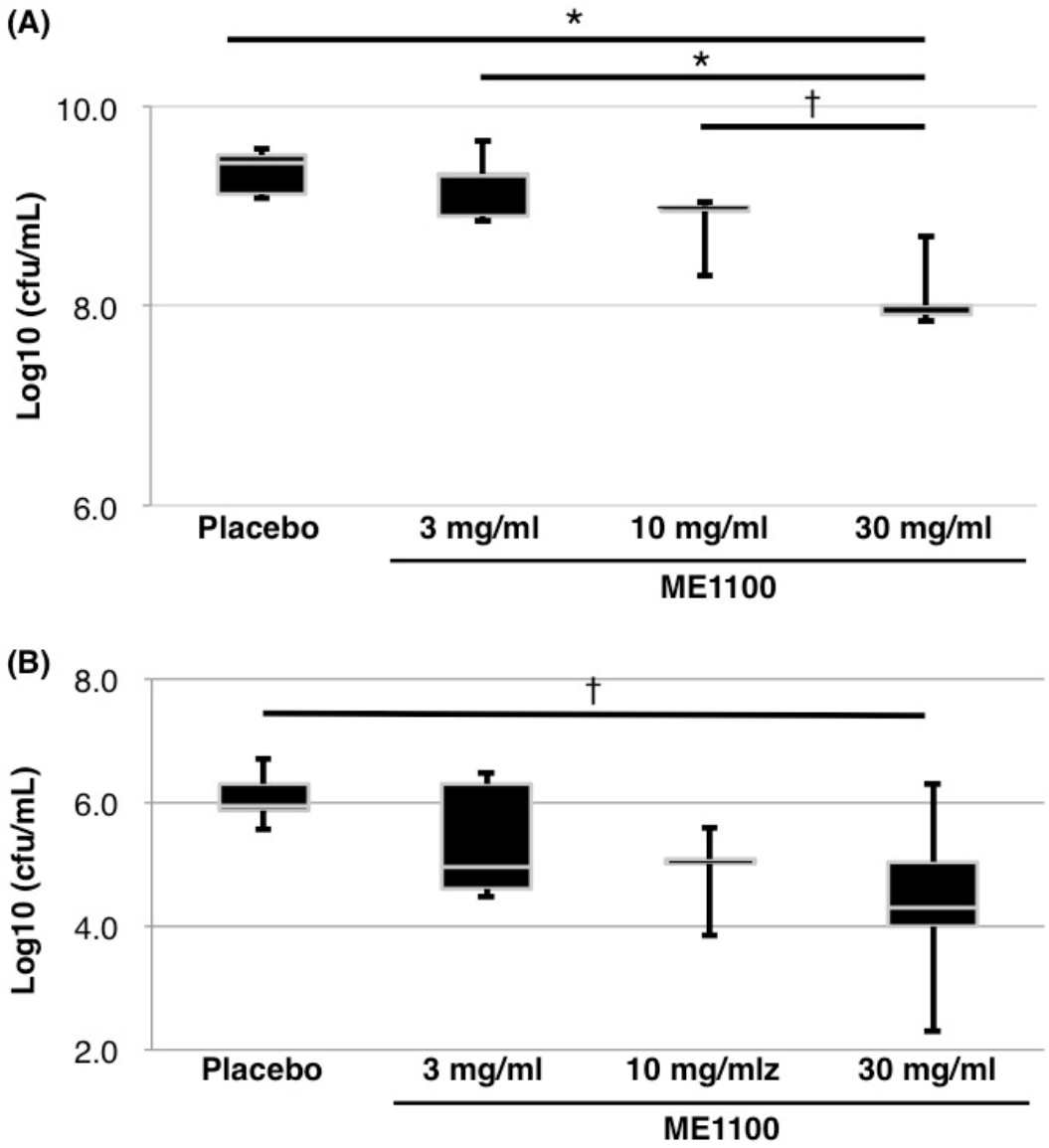

Fig. 2. Bacterial count in the lungs and blood.

Twelve hours post inoculation, the mice were treated for 5 minutes with placebo, 3, 10, or 30 mg/mL of ME1100. Six hours after the initial treatment, the mice were sacrificed, and the number of bacteria in the lungs (A) and the blood (B) was analysed ($n = 5$ in each groups). Box-and-whisker plots showing the range and median of the bacterial count. The bacterial count in the lungs was significantly decreased in the 30 mg/mL ME1100 treatment group, compared with all other groups (* $P < 0.001$ versus the placebo and 3 mg/mL ME1100; † $P < 0.05$, versus the 10 mg/mL of ME1100). The bacterial count in the blood of the mice treated with 30 mg/mL of ME1100 was significantly decreased in comparison with the placebo group († $P < 0.05$).

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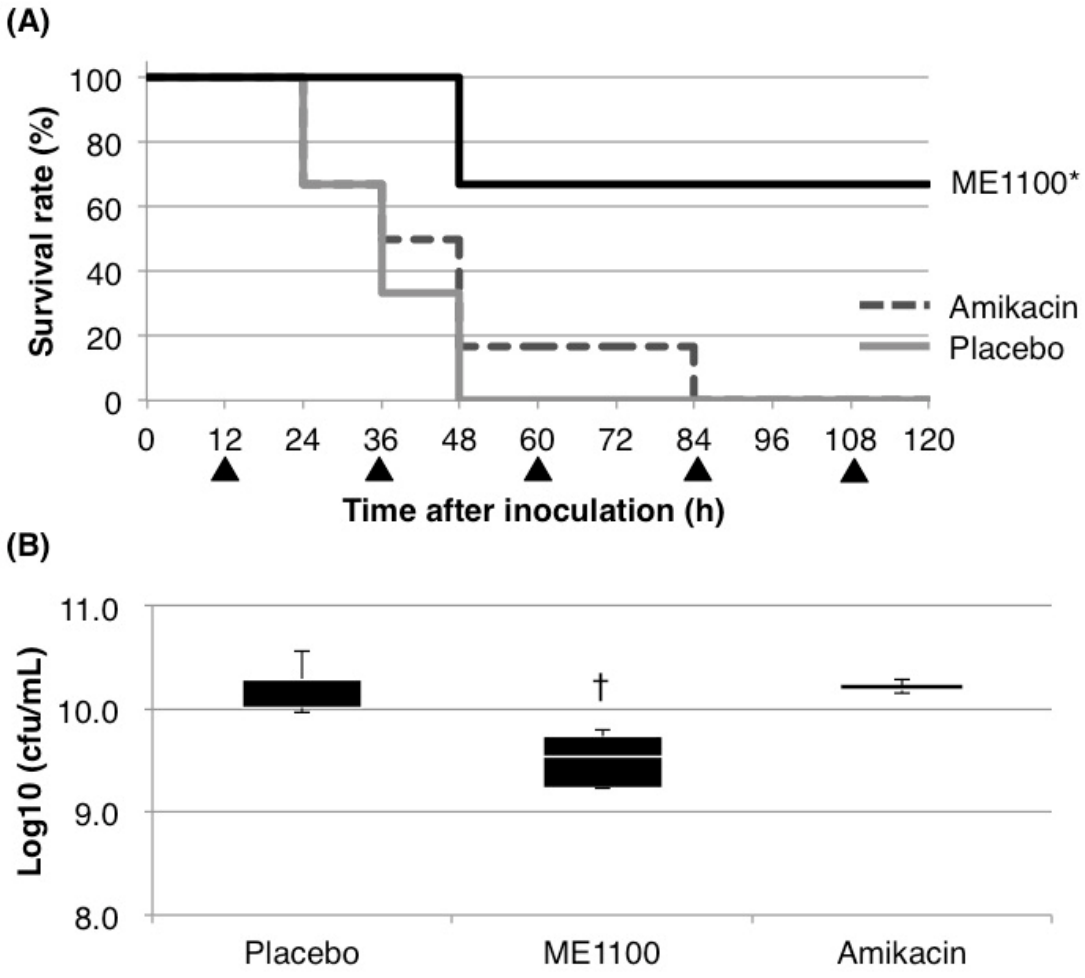

Fig. 3. Comparison of ME1100 and amikacin.

Twelve hours post inoculation, the mice were treated for 5 minutes, once daily, with placebo, 30 mg/mL of ME1100, or 30 mg/ml of amikacin. Survival was estimated at the indicated time, and the results are displayed as a Kaplan-Meier plot (A). The black triangles represent the time of treatment. The survival rates were observed for 120 hours after inoculation ($n = 6$ in each group). *The survival rates in the ME1100 treatment group was significantly higher than that in the placebo and the 30 mg/mL of amikacin treatment groups ($P < 0.005$, versus placebo and $P = 0.024$, versus amikacin).

Six hours after initial treatment, the mice were sacrificed, and the number of bacteria in the lungs was analysed ($n = 5$ in each groups). Box-and-whisker plots showing the range and median of the number of bacteria (B). The number of bacteria in the lungs was significantly decreased in the ME1100 treatment group, compared with the placebo and amikacin treatment groups ($^{\dagger}P < 0.001$ versus both groups).

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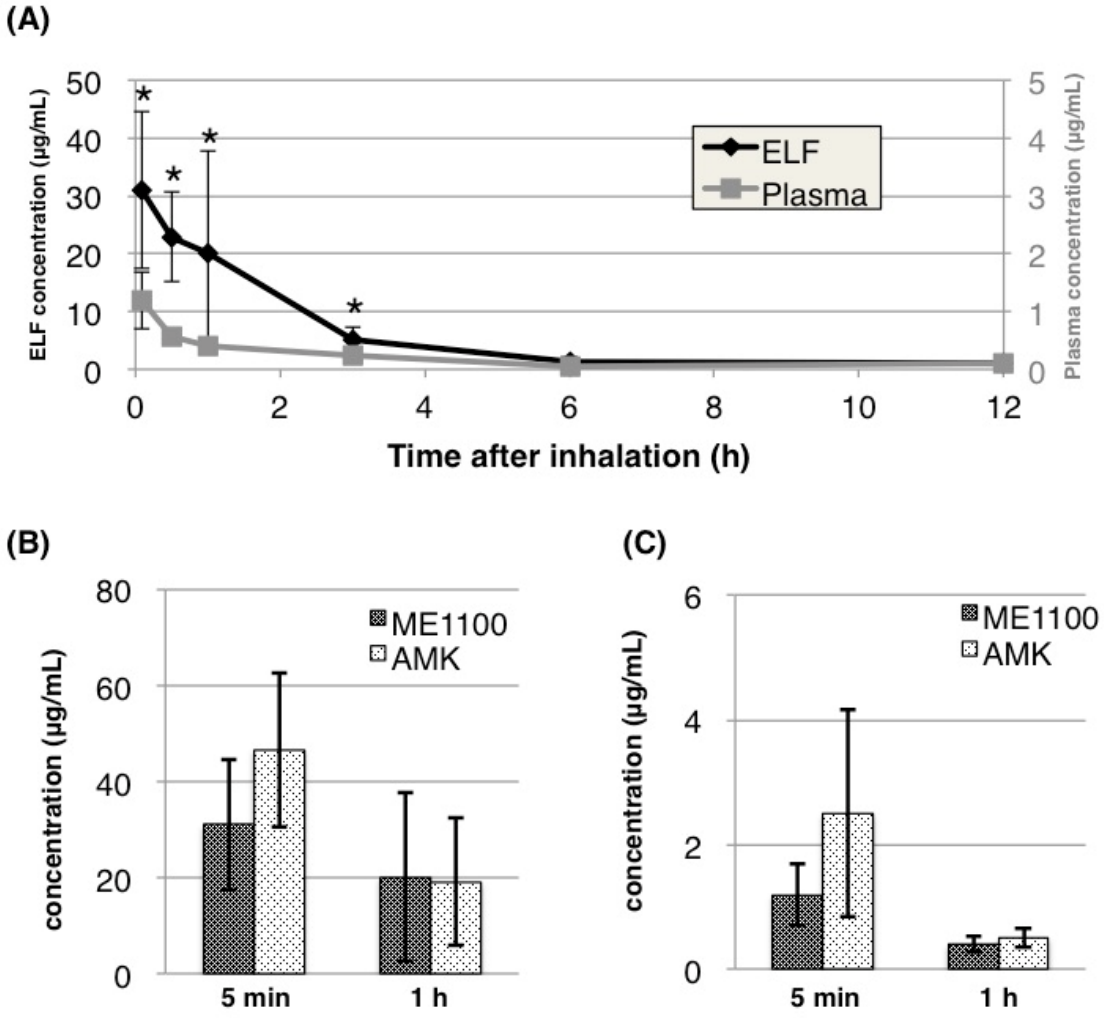

Fig. 4. ELF and Plasma concentrations of ME1100 and amikacin

The mice were treated for 5 minutes with placebo, 30 mg/mL of ME1100, or amikacin ($n = 4$ in each group). The pharmacokinetics of ME1100 in ELF (filled black rhombus) and plasma (filled gray square) were measured at 5, 30 minutes, 1, 3, 6, and 12 hours post inhalation (A). *The concentration of ME1100 in the ELF was significantly higher than that in the plasma for 3 hours post inhalation ($P = 0.028$). The concentrations of ME1100 and amikacin in ELF (B) and plasma (C) were compared at 5 minutes and 1 hour post inhalation. There were no significant differences between ME1100 and amikacin concentrations in both ELF and plasma.

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Figure legends:

Fig. 1. Therapeutic effects of ME1100 on the survival rate.

Twelve hours post inoculation, the mice were treated for 5 minutes, once daily, with placebo, 3, 10, or 30 mg/mL of ME1100. Survival was estimated at the indicated time, and the results are displayed as a Kaplan-Meier plot. The black triangles represent the time of treatment. The survival rates were observed for 120 hours after inoculation (n = 7 in each group). * The survival rates in the 30 mg/mL ME1100 treatment group was significantly higher than that in the placebo and 3 mg/mLME1100 treatment groups ($P <$ 0.001 *versus* placebo, and $P = 0.022$ *versus* the 3 mg/ mL of ME1100).† The survival rates in the 10 mg/mL ME1100 treatment group were significantly higher than those in the placebo group ($P < 0.001$).

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Six hours after initial treatment, the mice were sacrificed, and the number of bacteria in the lungs was analysed ($n = 5$ in each groups). Box-and-whisker plots showing the range and median of the number of bacteria (B). The number of bacteria in the lungs was significantly decreased in the ME1100 treatment group, compared with the placebo and amikacin treatment groups ($^{\dagger}P < 0.001$ versus both groups).

Fig. 4. ELF and plasma concentrations of ME1100 and amikacin

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