Efficacy of High-Dose Meropenem (6 g/day) in the Treatment of Experimental Murine Pneumonia Induced by Meropenem-resistant *Pseudomonas aeruginosa*

Running title: High-dose Meropenem for Murine Pneumonia

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ABSTRACT

High-dose meropenem (MEPM; 6 g/day) has been approved as a treatment for purulent meningitis; however, little is known regarding its *in vivo* efficacy in refractory lower respiratory tract infection. The purpose of this study was to evaluate the efficacy of 6 g/day MEPM in a murine model of severe pneumonia caused by MEPM-resistant *Pseudomonas aeruginosa*. Experimental pneumonia induced by MEPM-resistant *P. aeruginosa* was treated with normal-dose MEPM (150 mg/kg, simulating 3 g/day regimen in humans) or high-dose MEPM (500 mg/kg, simulating 6 g/day regimen in humans). Mice treated with high-dose MEPM showed significantly restored survival compared to that of untreated mice, and tended to show a higher survival rate compared to that of mice treated with normal-dose MEPM. The viable bacteria counts in the lungs significantly decreased in mice treated with high-dose MEPM compared with that of untreated mice ($P < 0.001$) and mice treated with normal-dose MEPM ($P < 0.01$ and $P < 0.05$). The number of inflammatory cells in the BALF was also significantly lower in mice treated with high-dose MEPM than in untreated mice. The free MEPM concentration in the epithelial lining fluid (ELF) exceeded 16 μg/mL for 85 min in mice treated with high-dose MEPM, but not in mice treated with normal-dose MEPM. Our results demonstrate that high-dose MEPM (6 g/day) might provide superior protection

against pneumonia caused by MEPM-resistant strains of *P. aeruginosa* compared to that by the normally administered dose (less than 3 g/day).

Introduction

Pseudomonas aeruginosa is one of the major causes of hospital-acquired pneumonia (HAP) and opportunistic infection (1). HAP and bacteremia caused by *P. aeruginosa* can be fatal. A mortality rate of 47% for *P. aeruginosa* pneumonia patients has been reported. Mortality as a result of *P. aeruginosa* pneumonia is associated with a delay in initiating effective antimicrobial therapy and multidrug-resistant *P. aeruginosa* (2,3).

MEPM is an antimicrobial drug that is potent against *P. aeruginosa*; however, the incidence of multidrug-resistant *P. aeruginosa* (MDRP) has increased recently (4), and *P. aeruginosa* is the most common multidrug-resistant bacterial cause of hospital-acquired pneumonia and ventilator-associated pneumonia (1). Approximately 13% of all healthcare-associated *P. aeruginosa* infections in the United States are caused by MDRP (5). Although MDRP is responsible for only 0.6% of *P. aeruginosa* infections detected in Japan, MEPM-resistant *P. aeruginosa* and imipenem-resistant *P. aeruginosa* are responsible for 13.8% and 16.3% of *P. aeruginosa* infections, respectively, indicating an increase in the rate of infection by carbapenem-resistant *P. aeruginosa* (6). Carbapenem-resistant *P. aeruginosa* infections generally have a poorer prognosis than carbapenem-sensitive *P. aeruginosa* infections do (7). In addition, the efficacy of colistin against carbapenem-resistant *P. aeruginosa* infections has not been established

(8). Appropriate use of antimicrobial drugs is required to control the emergence of drug-resistant bacterial pathogens (9,10). Pharmacokinetics (PK) parameters, such as time above MIC (TAM), C_{max}/MIC, and AUC/MIC, are used to predict the effectiveness of antimicrobial drugs. However, PK parameters used as efficacy indicators differ among antimicrobial drugs (11,12). TAM is an efficacy indicator for carbapenems, including MEPM (11). Because the mutant prevention concentration and mutant selection window influence the emergence of drug-resistant bacterial pathogens, high doses of antibiotics can minimize the emergence of drug-resistant bacterial pathogens (13,14). In addition, high doses of antibiotics can lead to better outcomes compared to that observed with normal doses of antibiotics (15,16).

Several reports have shown the efficacy of high-dose antibacterial drugs against severe pneumonia. The efficacy of 3 g /day sulbactam/ampicillin for 4 days on intermediate-to-severe community-acquired pneumonia has been reported (17). High-dose tigecycline and colistin are effective against pneumonia caused by carbapenem-resistant *Klebsiella pneumonie* in liver transplant patients (18). While the indications of high-dose MEPM (6 g/day) are currently limited to purulent meningitis and cystic fibrosis (CF), high-dose MEPM (6 g/day) is used for acute exacerbation of CF. In addition, high-dose MEPM (6 g/day) reduces the sputum bacterial burden and

94 improves clinical status (19). High-dose MEPM (6 g/day) might be effective against
95 pneumonia caused by MEPM-resistant strains because high drug concentrations reach
96 the lungs.

97 This study was designed to evaluate the efficacy of high-dose MEPM (6 g/day) in
98 comparison to that of normal-dose MEPM as a treatment for severe pneumonia caused
99 by MEPM-resistant and low susceptibility *P. aeruginosa* in mice.

100

101 **MATERIALS AND METHODS**

102 **Bacterial isolates**

103 Clinical isolates of MEPM-resistant (MIC 16 μg/ml) *P. aeruginosa* were utilized in
104 this study. MEPM MIC was determined by broth microdilution method according to
105 CLSI guidelines. Isolate stored in trypticase soy broth with 10% glycerol stocks
106 maintained at -80°C at Nagasaki University Hospital was spread on LB agar (SIGMA,
107 Tokyo) and incubated overnight at 37°C under 5% CO_2 prior to use in the experiments.
108 The mechanism of MEPM resistance was not investigated in this study; however, we
109 confirmed that these strains did not produce metallo-beta-lactamase.

110

111 **Laboratory animals**

112 Pathogen–free, ddY mice (7 weeks old, female) weighing about 30 g were
113 purchased from SLC Inc., Shizuoka, Japan. All of the animals were housed in a
114 pathogen-free environment and received sterile food and water at laboratory of the
115 Animal Center for Biomedical Science at Nagasaki University (Nagasaki, Japan). The
116 Ethics Review Committee for Animal Experimentation at Nagasaki University approved
117 all experimental protocols used in this study.

118

119 **Pharmacokinetic (PK) studies and determination of dosing regimen**

120 Plasma concentrations of MEPM were measured after intraperitoneal administration
121 of 100 mg/kg MEPM combined with 100 mg/kg cilastatin in mice, and those in humans
122 after intravenous administration of 1 g of MEPM over 30 minutes were obtained from
123 previous data (20,21). The PK parameters were calculated using a two-compartment
124 model with the MULTI program (22). The percent time that free-drug concentrations
125 remained above the MIC (fTAM) was calculated using the PK parameters, protein
126 binding, and MIC. The level of protein binding of MEPM in mice is 10% (23). Because

127 the plasma protein binding of MEPM in human plasma is very low (2%) (24), total
128 plasma concentrations in humans were used as free-drug concentrations. Table 1 shows
129 the fTAM of the MEPM regimen for humans and mice. The fTAM of the MEPM for
130 humans was calculated when 1 g or 2 g of MEPM was administered to humans for 30
131 minutes, three times a day. Four-dose intraperitoneal administration of MEPM (10
132 ml/kg/dose) at 2-hour intervals was chosen to alleviate the pain of the mice. The dose
133 regimens for mice infected with the MEPM-resistant strain to achieve fTAM using
134 human regimens of 3 g/day and 6 g/day were 150 mg/kg×4/day and 500 mg/kg×4/day,
135 respectively (Table 1). The half-life of meropenem/cilastatin in mice is short (12
136 minutes) and plasma concentrations of meropenem/cilastatin at 2 h after administration
137 were less than 1/500 compared to that at 5 minutes after administration. Therefore, the
138 PK of the first dose of meropenem was equal to that of other doses of meropenem, and
139 TAM in the regimen with 2-hour intervals was the same as that in the regiment with
140 6-hour intervals.

141

142 **Murine models of pneumonia caused by *P. aeruginosa***

143 *P. aeruginosa* was cultured on LB agar and incubated overnight at 37°C under 5%
144 CO_2, and the organisms were suspended in normal saline. For the pneumonia with

145 bacteremia study, 20 μl of the suspended MEPM-resistant strain (clinical isolate 1:
146 3×10^7 CFU and clinical isolate 2: 1×10^8 CFU) was inoculated intranasally with
147 anesthesia. For the pneumonia study, 20 μl of the suspended MEPM-resistant strain
148 (clinical isolate 1: 3×10^6 CFU and clinical isolate 2: 1×10^7 CFU) was inoculated
149 intranasally with anesthesia. MEPM was administered intraperitoneally 3 h after
150 inoculation in the pneumonia with bacteremia model and 14 h after inoculation in the
151 pneumonia model. MEPM at 150 mg/kg and 500 mg/kg was administered 4 times/day
152 at 2-hour intervals in combination with 100 mg/kg cilastatin to yield PK similar to that
153 in humans (3 g/day and 6 g/day, respectively). The treatment lasted for 2 days and 1 day
154 in the pneumonia with bacteremia model and in the pneumonia model, respectively. The
155 pneumonia with bacteremia model was used to evaluate the survival rate and the viable
156 bacterial counts in blood, whereas the pneumonia model was used to evaluate the viable
157 bacterial counts in the lungs, the number of inflammatory cells in BALF, free drug
158 concentrations of MEPM in epithelial lining fluid (ELF), and for histopathological
159 analysis of the lungs.
160
161 **Lung preparation for CFU determination and histopathological analysis**

Whole lungs were removed under aseptic conditions and homogenized in 1.0 ml phosphate-buffered saline (PBS). *P. aeruginosa* was quantified by placing serial dilutions of the lung homogenates onto LB agar plates and incubating them at 37°C in a 5% CO_2 atmosphere. For histopathological analysis, lung specimens were fixed in 10% formalin-buffered solution, and then the lung tissue sections were paraffin embedded and stained with hematoxylin and eosin (HE) using standard procedures (25).

Bronchoalveolar lavage fluid (BALF) cell analysis

BAL analysis was performed with different mice from the mice used for CFU determination and histopathological analysis to assess inflammatory cell accumulation in the airspace. The chest was opened to expose the lungs after the mice were anesthetized, and a disposable sterile feeding tube (Toray Medical Co., Chiba, Japan) was inserted into the trachea. BAL was performed using 1.0 ml of PBS, and the recovered fluid was pooled for each mouse. Total cell counts were performed by Turk staining with a hemacytometer (25,26).

Measurement of MEPM concentrations in ELF

BALF samples were mixed with 4 volumes of methanol, vortex mixed, and

centrifuged at 10,000 g for 10 min at 4°C. The supernatants were stored at -80°C until the measurement of MEPM concentrations by HPLC. The supernatants (50 μl) were separated on a Xterra MS C_{18} reverse phase column (3.5 μm, 4.6 × 20 mm; Nihon Waters K.K., Tokyo, Japan) with methanol-5 mM sodium dihydrogenphosphate (pH 7.0) (3:17) as the mobile phase delivered at 1.0 ml/min. The HPLC system (LC-2010C; Shimadzu Co., Kyoto, Japan) was controlled by a CLASS-VP workstation (Shimadzu), and the wavelength for MEPM detection was 300 nm. Five-point standard curves (0.1–10 μg/ml) were linear with $r^2 > 0.98$. The lower limit of quantitation was 0.1 μg/ml. The MEPM concentrations in ELF were calculated using the following formula: concentration in ELF = concentration in BALF × (urea in serum/urea in BALF). The fTAM in ELF was calculated as described above. Serum samples were also collected just before BAL was obtained from the same mice used for the urea assay.

Urea assay

The rate of decline of NADH levels induced by NH3 in the samples was measured. Urea was hydrolyzed by urease to produce NH3. The produced NH3 reacted with α-ketoisohexanoic acid and NADH by the action of leucine dehydrogenase to form leucine and NAD. The rate of decline of NADH levels at this point was measured

optically, and the urea content in the sample was calculated by subtracting the rate of decline resulting from the endogenous ammonia reaction.

Statistical analysis

All data were analyzed by using Prism 5 GraphPad Software and expressed as the mean ± standard error of the mean (SEMs). Survival analysis was performed using the log-rank test, and the survival rate was calculated by the Kaplan-Meier method. Differences between groups were examined using the Kruskal-Wallis test and Dunn's Multiple Comparison Test. $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

High-dose MEPM treatment protects mice from pneumonia induced by MEPM-resistant *P. aeruginosa*

Survival of the mice was observed for 7 days after infection. As shown in Fig. 1, the survival of mice treated with high-dose MEPM was significantly restored compared with that of untreated mice in the MEPM-resistant strain-induced pneumonia and bacteremia model. In addition, the survival of mice treated with high-dose MEPM was

higher than that of mice treated with normal-dose MEPM; however, no significant difference was observed.

Superior bactericidal activity of high-dose MEPM compared to that of the normal dose in the blood and lungs

The viable bacteria counts in blood were evaluated 4 h after infection (1 h after the first dose of MEPM) in the MEPM-resistant strain-induced pneumonia and bacteremia model (Fig. 2A,C). The viable bacteria counts in blood significantly decreased in the 500 mg/kg × 4/day group compared to those in both the untreated and 150 mg/kg × 4/day groups [clinical isolate 1: 500 mg/kg × 4/day group vs. untreated group = (1.72 ± 0.12 vs. 4.37 ± 0.17) log cfu/ml, $P < 0.001$, clinical isolate 2: 500 mg/kg × 4/day group vs. untreated group = (1.97 ± 0.23 vs. 4.23 ± 0.14) log cfu/ml, $P < 0.001$) and [clinical isolate 1: 500 mg/kg × 4/day group vs. 150 mg/kg × 4/day group = (1.72 ± 0.12 vs 3.27 ± 0.32) log cfu/ml, $P < 0.05$]. However, there was no significant difference between the untreated and the 150 mg/kg × 4/day groups. The viable bacteria counts in the lungs were evaluated 36 h after infection (24 h after 1st dose of MEPM) in the pneumonia model (Fig. 2B,D). The viable bacteria counts in the lungs significantly decreased in the 500 mg/kg × 4/day group compared to those in both the untreated and 150 mg/kg ×

4/day groups [clinical isolate 1: 500 mg/kg × 4/day group vs. untreated group = (2.61±0.33 vs. 5.11 ± 0.30) log cfu/ml, $P < 0.001$, clinical isolate 2 : 500 mg/kg × 4/day group vs. untreated group = (3.56±0.15 vs. 5.28 ± 0.19) log cfu/ml, $P < 0.001$] and [clinical isolate 1: 500 mg/kg × 4/day group vs. 150mg/kg × 4/day group = (2.61 ± 0.33 vs 4.28 ± 0.31) log cfu/ml, $P < 0.01$, clinical isolate 2 : 500 mg/kg × 4/day group vs. 150mg/kg × 4/day group = (3.56±0.15 vs. 4.41 ± 0.15) log cfu/ml, $P < 0.05$]. However, there was no significant difference between the untreated and the 150 mg/kg × 4/day groups.

High-dose MEPM treatment inhibits the pulmonary inflammation induced by MEPM-resistant *P. aeruginosa*

Lung quantitative cultures and BALF granulocyte counts over time in the control group (Fig. 3A,B) was evaluated. The number of bacteria in the lungs and BALF granulocytes at the evaluation point (38 h after infection) were higher than the numbers at the previous 2 time points (6 and 14 h after infection). The number of inflammatory cells in the BALF (Fig. 3C) was evaluated 38 h after infection in the pneumonia model. The number of inflammatory cells in the BALF significantly decreased in the 500 mg/kg × 4/day group compared to that in the untreated group [500 mg/kg × 4/day group vs.

252 untreated group = (5.44 ± 0.11 vs. 6.13 ± 0.13) log cells/ml, $P < 0.01$]. However, there
253 was no difference between the 150 mg/kg × 4/day and untreated groups.
254
255 **Histopathological examination**
256 As shown in Fig 4, histopathological analysis of the lungs stained with HE at 38 h after
257 infection revealed that the 500 mg/kg × 4/day treatment was more effective than the 150
258 mg/kg × 4/day treatment was.
259
260 **The kinetics of free drug concentrations in the ELF of mice administered high-dose**
261 **MEPM**
262 Free drug concentrations of MEPM in ELF were evaluated in both infected and
263 uninfected mice and were found to not exceed 16 μg/ml even when a dose of 500 mg/kg
264 was administered to uninfected mice (Fig. 4A). Conversely, in infected mice, the free
265 drug concentrations exceeded 16 μg/ml for 85 min after administration at 500 mg/kg;
266 however, it never exceeded 16 μg/ml after administration of MEPM at 150 mg/kg. The
267 fTAMs of infected mice in the 500 mg/kg × 4/day and 150 mg/kg × 4/day groups were
268 23.6% and 0%, respectively (Fig. 4B). These data suggest that increased penetration in
269 the airway by high-dose MEPM might underlie the protection against MEPM-resistant *P.*

aeruginosa pulmonary infection.

DISCUSSION

The efficacy of β-lactam drugs, including MEPM, is generally predicted by comparison between MIC for causative bacteria and unbound drug concentration in the extracellular fluid. The efficacy of MEPM can be discussed by considering the unbound drug concentration in the extracellular fluid (intracellular substance) as the total plasma concentration because the plasma protein binding rate of MEPM in humans is as low as 2%. In contrast, the plasma protein binding rate of MEPM is as high as 10% in mice; thus, the efficacy of MEPM should be discussed by taking this difference into consideration. In addition, a number of reports have suggested that the antibacterial drug concentration in the topical infected site reflects the efficacy of the drug. Although the topical infected site is indicative of intracellular substances in the lungs in pneumonia because of the presence of extracellular respiratory tract pathogens, including *P. aeruginosa*, bacteria can exist on the alveolar surface as well. Thus, the drug concentration in the ELF is also an important factor that needs to be considered when discussing therapeutic efficacy (27-29). Hence, we measured the changes in MEPM concentration in ELF over time to evaluate its association with therapeutic efficacy.

A non-infection mouse model and a mouse model of *P. aeruginosa* infection were used to measure the MEPM concentration in the ELF. Because the migration of MEPM into the ELF was found to be poor in the non-infection model, the unbound MEPM concentration in ELF did not exceed 16 μg/ml in both the 150 mg/kg × 4/day group and 500 mg/kg × 4/day group. In the *P. aeruginosa* infection model, however, the unbound MEPM concentration in the ELF did not exceed 16 μg/ml in the 150 mg/kg × 4/day group and fTAM was 0%, whereas fTAM was 23.6% in the 500 mg/kg × 4/day group. Carbapenems, including MEPM, have been reported to exert a bacteriostatic effect at TAM of 20% to 30% and an antimicrobial effect at TAM of 40% to 50% (30,31). The TAM in the 500 mg/kg × 4/day group was 23.6%, supporting the following effects of MEPM in the 500 mg/kg × 4/day group: trend toward improved survival rate, significant reduction of viable bacteria counts in the lungs, and improvement of inflammatory cell infiltration in the lungs in comparison with that in BALF and as observed by pathological images. In this study, a trend toward improved survival rate and reduction of viable bacteria counts in the lungs was observed in the 150 mg/kg × 4/day group compared to those observed in the untreated group. The following factors may have potentially contributed to the reduction of viable bacteria counts in the lungs and improvement of survival rate: the plasma MEPM concentration achieved a TAM of

306 17.2%, a certain antimicrobial effect of MEPM was obtained at the sub-MIC level (32),
307 and MEPM itself enhanced phagocytosis of bacteria by macrophages (33). The results
308 of this study indicate that high-dose MEPM (6 g/day) was more effective against
309 pneumonia caused by MEPM-resistant *P. aeruginosa*. *P. aeruginosa* is a typical
310 causative bacteria in HAP and healthcare-associated pneumonia with high mortality (1).
311 Because delayed administration of effective antimicrobial therapy and failure of initial
312 therapy result in poor prognosis, clinicians should select antibacterial drugs and initiate
313 therapy without waiting for drug sensitivity test results for treatment of patients
314 suspected to have *P. aeruginosa* pneumonia. Carbapenem antibiotics, including MEPM,
315 are first-line drugs for treatment of *P. aeruginosa* pneumonia. In this study, the drug
316 concentration at the topical infection site (both plasma and ELF) of MEPM-resistant *P.*
317 *aeruginosa* (MIC of MEPM = 16 μg/ml) was unsatisfactory on administration of
318 normal-dose MEPM (3 g/day), and this may have potentially led to treatment failure.
319 However, high-dose MPEM (6 g/day) achieved TAM (both plasma and ELF), which
320 enabled a sufficient treatment effect against MEPM-resistant *P. aeruginosa* (MIC of
321 MEPM =16 μg/ml). The MIC_{90} of MEPM against *P.aeruginosa* is equal to or less than
322 16 μg/ml in Japan and the other countries (34-41); thus, according to PK-PD theory,
323 high dose MEPM (6g/day) might be able to achieve a clinical efficacies greater than

90% against *P. aeruginosa*. In clinical practice, high-dose MEPM may be indicated in patients suspected to have severe *P. aeruginosa* pneumonia for whom failure of initial therapy is not permissible. Moreover, high-dose (6 g/day) MEPM may be highly effective in patients with MEPM-sensitive *P. aeruginosa* pneumonia and in immunocompromised patients with neutropenia, lung abscess, cystic fibrosis, or idiopatic pulmonary fibrosis (IPF) that limits drug migration into the topical infected site. Therefore, further validation, including clinical studies, are necessary.

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REFERENCES

1. **Chalmers JD, Rother C, Salih W, Ewig S**. 2014. Healthcare-associated pneumonia does not accurately identify potentially resistant pathogens: a systematic review and meta-analysis. Clin Infect Dis **58**(3):330–339.

2. **Tumbarello M, De Pascale G, Trecarichi EM, Spanu T, Antonicelli F, Maviglia R, Pennisi MA, Bello G, Antonelli M.** 2013. Clinical outcomes of Pseudomonas aeruginosa pneumonia in intensive care unit patients. Intensive Care Med **39**(4):682–692.

3. **Kang CI, Kim SH, Kim HB, Park SW, Choe YJ, Oh MD, Kim EC, Choe KW**. 2003. Pseudomonas aeruginosa bacteremia: risk factors for mortality and influence of delayed receipt of effective antimicrobial therapy on clinical outcome. Clin Infect Dis **37**(6):745–751.

4. **American Thoracic S, Infectious Diseases Society of A**. 2005. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Resp Crit Care Med **171**(4):388–416.

5. **Solomon SL, Oliver KB**. 2014. Antibiotic resistance threats in the United States: stepping back from the brink. Am Fam Physician **89**(12):938–941.

6. **Yanagihara K, Kadota J, Aoki N, Matsumoto T, Yoshida M, Yagisawa M, Oguri T, Sato J, Ogasawara K, Wakamura T, Sunakawa K, Watanabe A, Iwata S,**

Kaku M, Hanaki H, Ohsaki Y, Watari T, Toyoshima E, Takeuchi K, Shiokoshi M, Takeda H, Miki M, Kumagai T, Nakanowatari S, Takahashi H, Utagawa M, Nishiya H, Kawakami S, Kobayashi N, Takasaki J, Mezaki K, Konosaki H, Aoki Y, Yamamoto Y, Shoji M, Goto H, Saraya T, Kurai D, Okazaki M, Niki Y, Yoshida K, Kawana A, Saionji K, Fujikura Y, Miyazawa N, Kudo M, Sato Y, Yamamoto M, Yoshida T, Nakamura M, Tsukada H, Imai Y, Tsukada A, Kawasaki S, Honma Y, Yamamoto T, Ban N, Mikamo H, Sawamura H, Miyara T, Toda H, Sato K, Nakamura T, Fujikawa Y, Mitsuno N, Mikasa K, Kasahara K, Sano R, Sugimoto K, Asari S, Nishi I, Toyokawa M, Miyashita N, Koguchi Y, Kusano N, Mihara E, Kuwabara M, Watanabe Y, Kawasaki Y, Takeda K, Tokuyasu H, Masui K, Negayama K, Hiramatsu K, Aoki Y, Fukuoka M, Magarifuchi H, Nagasawa Z, Suga M, Muranaka H, Morinaga Y, Honda J, Fujita M. 2015. Nationwide surveillance of bacterial respiratory pathogens conducted by the surveillance committee of Japanese Society of Chemotherapy, the Japanese Association for Infectious Diseases, and the Japanese Society for Clinical Microbiology in 2010: General view of the pathogens' antibacterial susceptibility. J Infect Chemother **21**(6):410–420.

7. **Lee CH, Su TY, Ye JJ, Hsu PC, Kuo AJ, Chia JH, Lee MH**. 2015. Risk factors and clinical significance of bacteremia caused by Pseudomonas aeruginosa

resistant only to carbapenems. J Microbiol Immunol Infect. pii: S1684-1182(15)00767-7.

8. **Montero M, Horcajada JP, Sorli L, Alvarez-Lerma F, Grau S, Riu M, Sala M, Knobel H**. 2009. Effectiveness and safety of colistin for the treatment of multidrug-resistant Pseudomonas aeruginosa infections. Infection **37**(5):461–465.

9. **Luyt CE, Brechot N, Trouillet JL, Chastre J**. 2014. Antibiotic stewardship in the intensive care unit. Crit Care **18**(5):480.

10. **MacDougall C, Polk RE.** 2005. Antimicrobial stewardship programs in health care systems. Clin Microbiol Rev **18**(4):638–656.

11. **Breilh D, Texier-Maugein J, Allaouchiche B, Saux MC, Boselli E**. 2013. Carbapenems. J Chemother **25**(1):1–17.

12. **Okada K, Kimura T, Mikamo H, Kasahara K, Seki M, Takakura S, Tokimatsu I, Ohmagari N, Takahashi Y, Matsumoto K, Igarashi M, Kobayashi M, Hamada Y, Mochizuki T, Kimura M, Nishi Y, Tanigawara Y, Takesue Y; Japanese Society of Chemotherapy; Japanese Society of Therapeutic Drug Monitoring**. 2014. Clinical practice guidelines for therapeutic drug monitoring of arbekacin: a consensus review of the Japanese Society of Chemotherapy and the Japanese Society of Therapeutic Drug Monitoring. J Infect Chemother **20**(1):1–5.

396 13. **Canton R, Morosini MI**. 2011. Emergence and spread of antibiotic resistance
397 following exposure to antibiotics. FEMS Microbiol Rev **35**(5):977–991.
398 14. **Li X, Wang L, Zhang XJ, Yang Y, Gong WT, Xu B, Zhu YQ, Liu W.** 2014.
399 Evaluation of meropenem regimens suppressing emergence of resistance in
400 Acinetobacter baumannii with human simulated exposure in an in vitro
401 intravenous-infusion hollow-fiber infection model. Antimicrob Agents Chemother
402 **58**(11):6773–6781.
403 15. **Falagas ME, Vardakas KZ, Tsiveriotis KP, Triarides NA, Tansarli GS**.
404 2014. Effectiveness and safety of high-dose tigecycline-containing regimens for the
405 treatment of severe bacterial infections. Int J Antimicrob Agents **44**(1):1–7.
406 16. **Nicasio AM, Eagye KJ, Nicolau DP, Shore E, Palter M, Pepe J, Kuti JL.**
407 2010. Pharmacodynamic-based clinical pathway for empiric antibiotic choice in patients
408 with ventilator-associated pneumonia. J Crit Care **25**(1):69–77.
409 17. **Kohno S, Tateda K, Mikamo H, Kadota J, Niki Y, Itamura R**. 2015.
410 Efficacy and safety of intravenous sulbactam/ampicillin 3 g 4 times daily in Japanese
411 adults with moderate to severe community-acquired pneumonia: a multicenter,
412 open-label, uncontrolled study. J Infect Chemother **21**(3):182–188.
413 18. **Ralston SL, Lieberthal AS, Meissner HC, Alverson BK, Baley JE,**

Gadomski AM, Johnson DW, Light MJ, Maraqa NF, Mendonca EA, Phelan KJ, Zorc JJ, Stanko-Lopp D, Brown MA, Nathanson I, Rosenblum E, Sayles S, 3rd, Hernandez-Cancio S, American Academy of P. 2014. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. Pediatrics 134:e1474-1502.

19. **Blumer JL, Saiman L, Konstan MW, Melnick D.** 2005. The efficacy and safety of meropenem and tobramycin vs ceftazidime and tobramycin in the treatment of acute pulmonary exacerbations in patients with cystic fibrosis. Chest **128**(4):2336–2346.

20. **Harada Y, Morinaga Y, Kaku N, Nakamura S, Uno N, Hasegawa H, Izumikawa K, Kohno S, Yanagihara K.** 2014. In vitro and in vivo activities of piperacillin-tazobactam and meropenem at different inoculum sizes of ESBL-producing Klebsiella pneumoniae. Clin Microbiol Infect **20**(11):O831–289.

21. **Nakashima M, Uematsu, T, Kanamaru, M**. 1992. Clinical phase I study of meropenem. Chemotherapy **40**: 258–275.

22. **Ikawa K, Morikawa N, Urakawa N, Ikeda K, Ohge H, Sueda T.** 2007. Peritoneal penetration of doripenem after intravenous administration in abdominal-surgery patients. J Antimicrob Chemother **60**(6):1395–1397.

23. **Mattie H, Zhang LC, van Strijen E, Sekh BR, Douwes-Idema AE.** 1997. Pharmacokinetic and pharmacodynamic models of the antistaphylococcal effects of

meropenem and cloxacillin in vitro and in experimental infection. Antimicrob Agents Chemother **41**(10):2083–2088.

24. DE W. Merrem package insert. AstraZeneca. 2013.

25. **Tanaka A, Nakamura S, Seki M, Fukudome K, Iwanaga N, Imamura Y, Miyazaki T, Izumikawa K, Kakeya H, Yanagihara K, Kohno S.** 2013. Toll-like receptor 4 agonistic antibody promotes innate immunity against severe pneumonia induced by coinfection with influenza virus and Streptococcus pneumoniae. Clin Vaccine Immunol *:***20**(7):977–985.

26. **Yanagihara K, Seki M, Cheng PW**. 2001. Lipopolysaccharide Induces Mucus Cell Metaplasia in Mouse Lung. Am J Resp Cell Mol Biol **24**(1): 66–73.

27. **Rodvold KA, George JM, Yoo L.** 2011. Penetration of anti-infective agents into pulmonary epithelial lining fluid: focus on antibacterial agents. Clin Pharmacokinet **50**(10): 637–664.

28. **Kiem S, Schentag JJ.** 2008. Interpretation of antibiotic concentration ratios measured in epithelial lining fluid. Antimicrob Agents Chemother **52**(1): 24–36.

29. **Frippiat F, Musuamba FT, Seidel L, Albert A, Denooz R, Charlier C, Van Bambeke F, Wallemacq P, Descy J, Lambermont B, Layios N, Damas P, Moutschen M**. 2015. Modelled target attainment after meropenem infusion in patients with severe

450 nosocomial pneumonia: the PROMESSE study. J Antimicrob Chemother **70**(1):207–216

451 30. **Drusano GL.** 2003. Prevention of resistance: a goal for dose selection for

452 antimicrobial agents. Clin Infect Dis **36**(Suppl 1):S42–50.

453 31. **Kuti JL, Dandekar PK, Nightingale CH, Nicolau DP**. 2003. Use of Monte

454 Carlo simulation to design an optimized pharmacodynamic dosing strategy for

455 meropenem. J Clin Pharmacol **43**(10):1116–1123.

456 32. **Drenjancevic D, Vranes J, Bedenic B, Sakic-Zdravcevic K.** 2007. In vitro

457 effect of subinhibitory concentrations of ceftazidime and meropenem on the serum

458 sensitivity of Pseudomonas aeruginosa strains. Coll Antropol **31**(1):221–225.

459 33. **Cuffini AM, Tullio V, Allocco A, Giachino F, Fazari S, Carlone NA.** 1993.

460 The entry of meropenem into human macrophages and its immunomodulating activity. J

461 Antimicrob Chemother **32**(5):695–703.

462 34. **Yamaguchi K, Ishii Y, Tateda K, Iwata M, Watanabe N, Shinagawa M,**

463 **Kayaba H, Kimura M, Suwabe A, Kaku M, Abe Y, Kanemitsu K, Taniguchi N,**

464 **Murakami M, Maesaki S, Kawamura T, Nomura F, Watanabe M, Kanno H,**

465 **Horiuchi H, Tazawa Y, Kondo S, Misawa S, Takemura H, Nakashima H, Matsuto T,**

466 **Fujimoto Y, Ishigo S, Gotoh H, Watanabe O, Yagi T, Shimaoka N, Mikamo H,**

467 **Yamagishi Y, Fujita N, Komori T, Ichiyama S, Kawano S, Nakayama A, Nakamura**

F, Kohno H, Fukuda S, Kusano N, Nose M, Yokozaki M, Onodera M, Murao K, Negayama K, Nishimiya T, Miyamoto H, Matsunaga A, Yoshimura H, Kohno S, Yanagihara K, Hiramatsu K. 2014. Nationwide surveillance of parenteral antibiotics containing meropenem activities against clinically isolated strains in 2012. Jpn J Antibiot **67**(2):73–107.

35. **Ishikawa K, Hamasuna R, Uehara S, Yasuda M, Yamamoto S, Hayami H, Takahashi S, Matsumoto T, Minamitani S, Kadota J, Iwata S, Kaku M, Watanabe A, Sunakawa K, Sato J, Hanaki H, Tsukamoto T, Kiyota H, Egawa S, Deguchi T, Matsumoto M, Tanaka K, Arakawa S, Fujisawa M, Kumon H, Kobayashi K, Matsubara A, Wakeda H, Amemoto Y, Onodera S, Goto H, Komeda H, Yamashita M, Takenaka T, Fujimoto Y, Tsugawa M, Takahashi Y, Maeda H, Onishi H, Ishitoya S, Nishimura K, Mitsumori K, Ito T, Togo Y, Nakamura I, Ito N, Kanamaru S, Hirose T, Muranaka T, Yamada D, Ishihara S, Oka H, Inatomi H, Matsui T, Kobuke M, Kunishima Y, Kimura T, Ichikawa T, Kagara I, Matsukawa M, Takahashi K, Mita K, Kato M, Okumura K, Kawanishi H, Hashimura T, Aoyama T, Shigeta M, Koda S, Taguchi K, Matsuda Y.** 2015. Japanese nationwide surveillance in 2011 of antibacterial susceptibility patterns of clinical isolates from complicated urinary tract infection cases. J Infect Chemother **21:**623-633.

36. **Flamm RK, Nichols WW, Sader HS, Farrell DJ, Jones RN**. 2016. In vitro activity of ceftazidime/avibactam against Gram-negative pathogens isolated from pneumonia in hospitalised patients, including ventilated patients. Int J Antimicrob Agents **47**(3):235–242.

37. **Flamm RK, Rhomberg PR, Jones RN, Farrell DJ.** 2015. In vitro activity of RX-P873 against Enterobacteriaceae, Pseudomonas aeruginosa, and Acinetobacter baumannii. Antimicrob Agents Chemother **59**(4): 2280–2885.

38. **Kanj SS, Whitelaw A, Dowzicky MJ**. 2014. In vitro activity of tigecycline and comparators against Gram-positive and Gram-negative isolates collected from the Middle East and Africa between 2004 and 2011. Int J Antimicrob Agents **43**(2):170–178.

39. **Li Y, Lv Y, Xue F, Zheng B, Liu J, Zhang J**. 2015. Antimicrobial resistance surveillance of doripenem in China. J Antibiot **68**(8):496–500.

40. **Sader HS, Rhomberg PR, Farrell DJ, Jones RN.** 2015. Arbekacin activity against contemporary clinical bacteria isolated from patients hospitalized with pneumonia. Antimicrob Agents Chemother **59**(6):3263–3270.

41. **Tato M, Garcia-Castillo M, Bofarull AM, Canton R, Group CS.** 2015. In vitro activity of ceftolozane/tazobactam against clinical isolates of Pseudomonas

aeruginosa and Enterobacteriaceae recovered in Spanish medical centres: Results of the CENIT study. Int J Antimicrob Agents **46**(5): 502–510

Figure legends

Figure 1. Survival of mice infected with 3×10^7 (clinical isolate 1) or 1×10^8 (clinical

522 isolate 2) CFU of MEPM-resistant *P. aeruginosa* treated with MEPM at 500 mg/kg or
523 150 mg/kg, or PBS (untreated group), four times/day (n = 9-10). Statistical differences
524 compared to the untreated group were determined by the Kaplan-Meier log-rank test. .
525 *$P < 0.05$, **$P < 0.01$ (versus untreated group).
526
527 **Figure 2**. Dose-dependent bactericidal effect of MEPM. Number of viable bacteria in
528 blood (A,C). Mice were inoculated with 3×10^7 (clinical isolate 1) or 1×10^8 (clinical
529 isolate 2) CFU of MEPM-resistant *P. aeruginosa*. At 4 h after infection (1 h after the
530 first dose of MEPM), mice treated with MEPM at 500 mg/kg and 150 mg/kg and
531 untreated mice were compared (A). At 5 h after infection (2 h after the first dose of
532 MEPM), the mice in each group were compared (C). The number of viable bacteria was
533 significantly lower in the 500 mg/kg treatment group (n=9). ***$P < 0.001$ (versus
534 untreated group), *$P < 0.05$ (versus 150 mg/kg treatment group). Number of viable
535 bacteria in the lungs (B,D). Mice were inoculated with 3×10^6 (clinical isolate 1) and
536 1×10^7 (clinical isolate 2) CFU of MEPM-resistant *P. aeruginosa*. At 38 h after infection
537 (24 h after 1st dose of MEPM), mice treated with MEPM at 500 mg/kg × 4/day and 150
538 mg/kg × 4/day and untreated mice were compared. The number of viable bacteria in the
539 lungs was significantly lower in the 500 mg/kg × 4/day treatment group ((B): n=13-15,

(D): n=10-11). ***$P < 0.001$ (versus untreated group), **$P < 0.01$, *$P < 0.05$ (versus 150 mg/kg treatment group).

Figure 3. Number of inflammatory cells in BALF. Mice were inoculated with 3×10^6 CFU of MEPM-resistant *P. aeruginosa*. At 38 h after infection (24 h after the first dose of MEPM), mice treated with MEPM at 500 mg/kg × 4/day and 150 mg/kg × 4/day and untreated mice were compared. Lung quantitative cultures and BALF granulocyte counts over time in the control group (A,B). The number of bacteria in the lungs and BALF granulocytes at the evaluation point (38 h after infection) were higher than the numbers at the previous 2 time points (6 and 14 h after infection). The number of inflammatory cells in BALF was significantly lower in the 500 mg/kg × 4/day treatment group (n=7)(C). **$P < 0.01$ (versus untreated group or 6h after infection).

Figure 4. Histopathological analysis of the lungs of mice inoculated with 3×10^6 CFU of MEPM-resistant *P. aeruginosa* and treated with high-dose MEPM. At 38 hours after infection (24 hour after the first dose of MEPM), mice treated with MEPM at 500 mg/kg × 4/day and 150 mg/kg × 4/day and mice with no treatment were compared. HE-stained tissue sections were observed at magnifications of ×40 and ×200.

No-treatment group (A), 150 mg/kg×4/day group (B), 500 mg/kg×4/day group (C). The inflammation of lungs decreased in a dose-dependent manner. The accumulation of inflammatory cells, hemorrhage in the lungs, and destruction of alveoli were limited in mice treated with MEPM at 500 mg/kg × 4/day.

Figure 5. Free drug concentrations of MEPM in ELF at 5, 15, 30, and 60 minutes after 4 doses of MEPM in non-infected mice (A) and mice infected with 3×10^6 CFU of MEPM-resistant *P. aeruginosa* (B). The free drug concentrations of MEPM in the ELF did not reach 16 μg/ml even when MEPM was administered at 500 mg/kg in non-infected mice (A). The free drug concentrations of MEPM in the ELF were higher than 16 μg/ml for 85 min in the mice treated with 500 mg/kg of MEPM; however, they never exceeded 16 μg/ml in the mice treated with 150 mg/kg of MEPM.

576 **Table 1**

Host	Dose regimen	fTAM* (%)
Human	2 g × 3/day	25.1
Mouse	500 mg/kg × 4/day	24.8
Human	1 g × 3/day	15.4
Mouse	150 mg/kg × 4/day	17.4

577 PK/PD parameters. fTAM of MEPM dose regimens for humans and mice with
578 pneumonia caused by MEPM-resistant *P. aeruginosa.* *fTAM: the percent time that
579 free-drug concentrations remain above the MIC.

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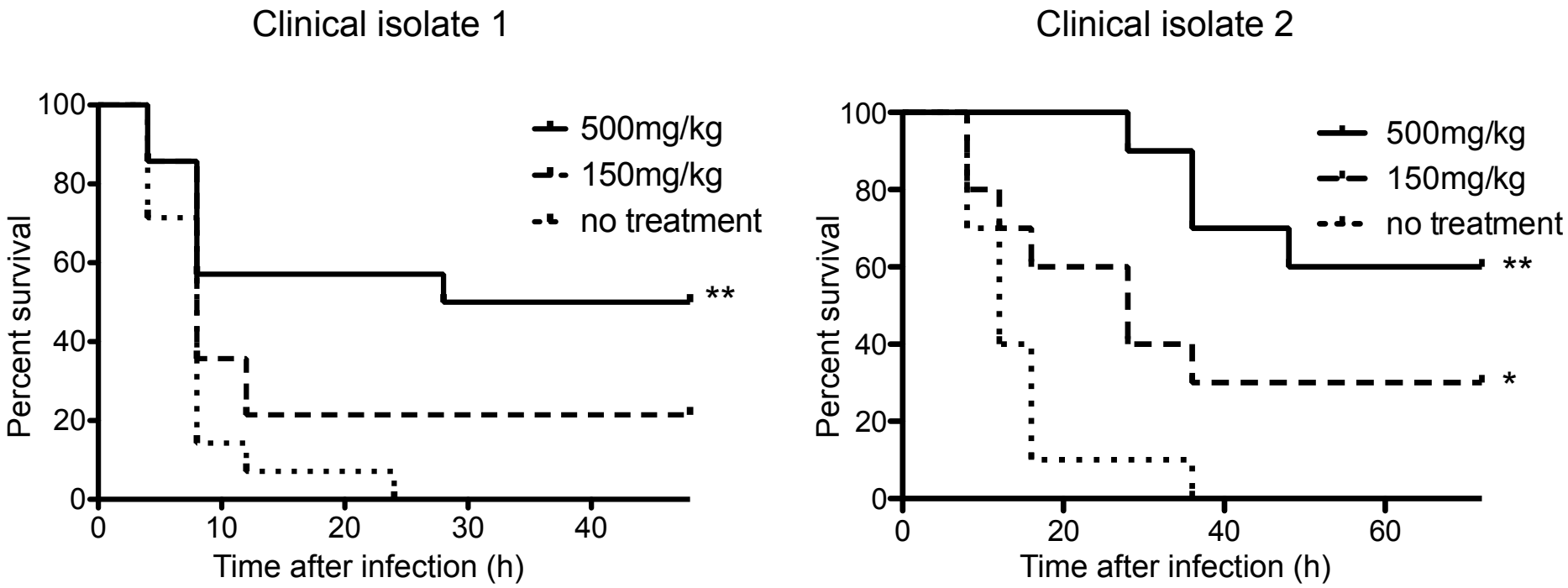


Figure 1. The survival of mice infected with 3×10^7 (clinical isolate 1) or 1×10^8 (clinical isolate 2) CFU of MEPM-resistant *P. aeruginosa* treated with MEPM at 500 mg/kg or 150 mg/kg, or PBS (untreated group), four times/day (n = 9-10). Statistical differences compared to untreated group were determined by the Kaplan-Meier log-rank test. $*P < 0.05$, $**P < 0.01$ (versus untreated group).

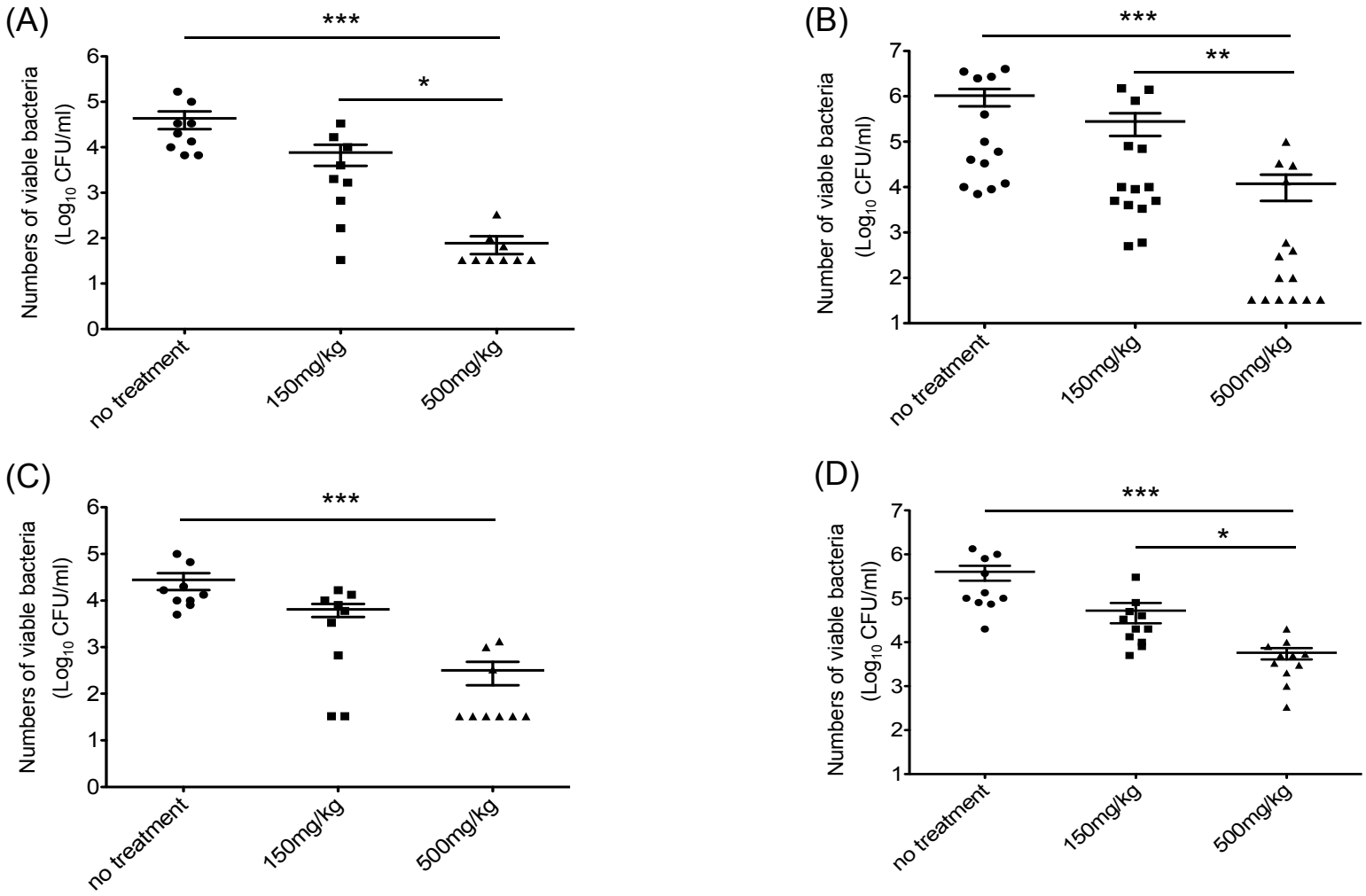
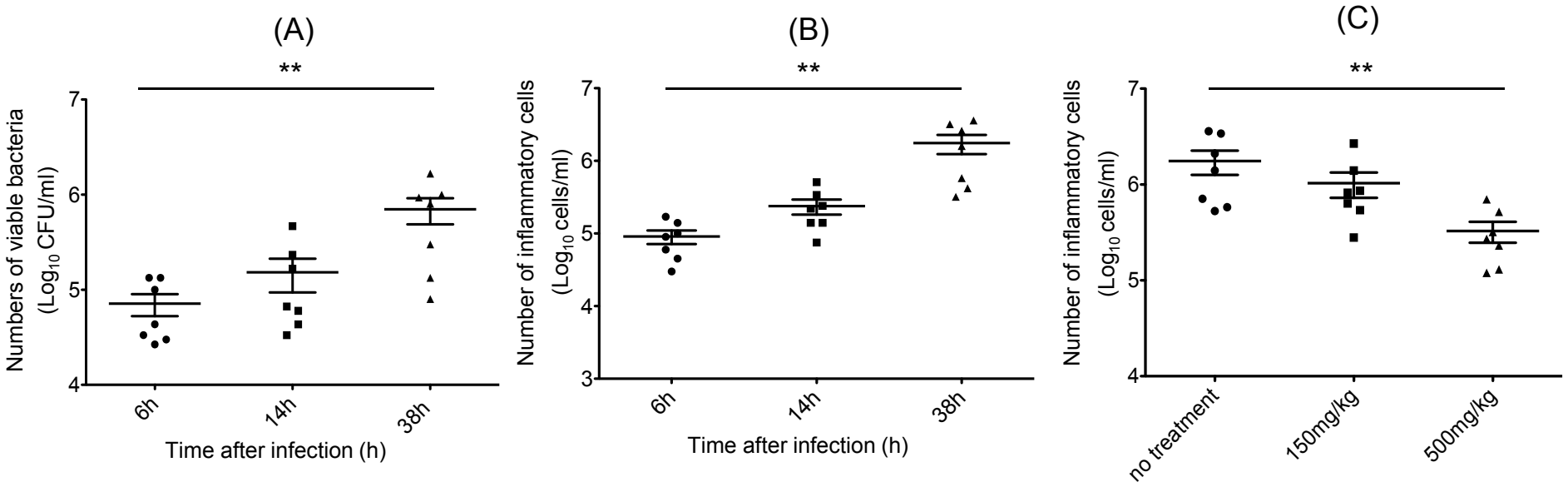


Figure 2. Dose-dependent bactericidal effect of MEPM. Number of viable bacteria in blood (A,C). Mice were inoculated with 3×10^7 (clinical isolate 1) or 1×10^8 (clinical isolate 2) CFU of MEPM-resistant *P. aeruginosa*. At 4 h after infection (1 h after 1st dose of MEPM), mice treated with MEPM at 500 mg/kg and 150 mg/kg and untreated mice were compared (A,C). The number of viable bacteria was significantly lower in the 500 mg/kg treatment group (n=9). ***$P < 0.001$ (versus untreated group), *$P < 0.05$ (versus 150 mg/kg treatment group). Number of viable bacteria in the lungs (B,D). Mice were inoculated with 3×10^6 (clinical isolate 1) and 1×10^7 (clinical isolate 2) CFU of MEPM-resistant *P. aeruginosa*. At 38 h after infection (24 h after 1st dose of MEPM), mice treated with MEPM at 500 mg/kg × 4/day and 150 mg/kg × 4/day and untreated mice were compared. The number of viable bacteria in the lungs was significantly lower in the 500 mg/kg × 4/day treatment group ((B):n=13-15, (D):n=10-11). ***$P < 0.001$ (versus untreated group), **$P < 0.01$, *$P < 0.05$ (versus 150 mg/kg treatment group).



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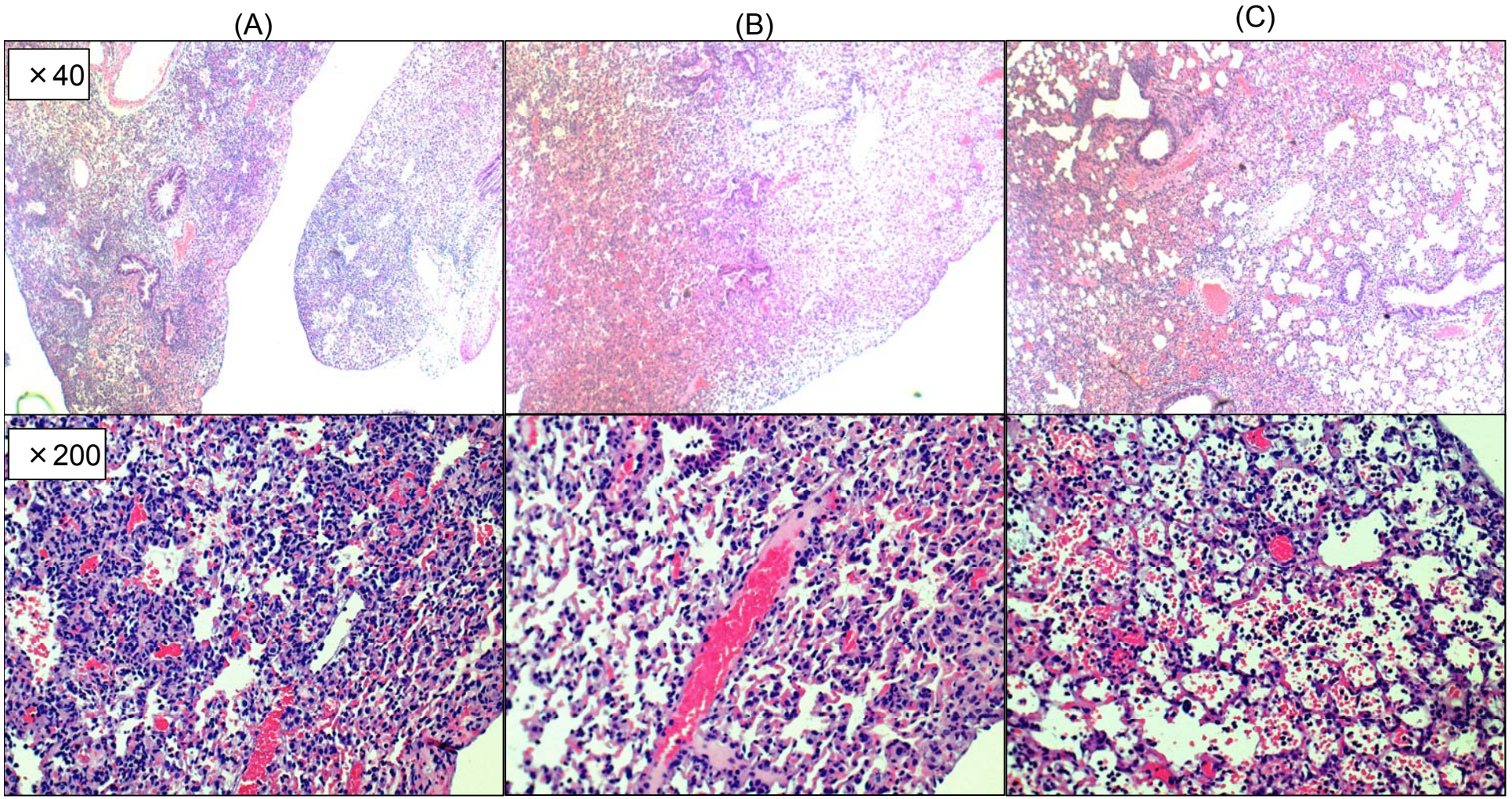
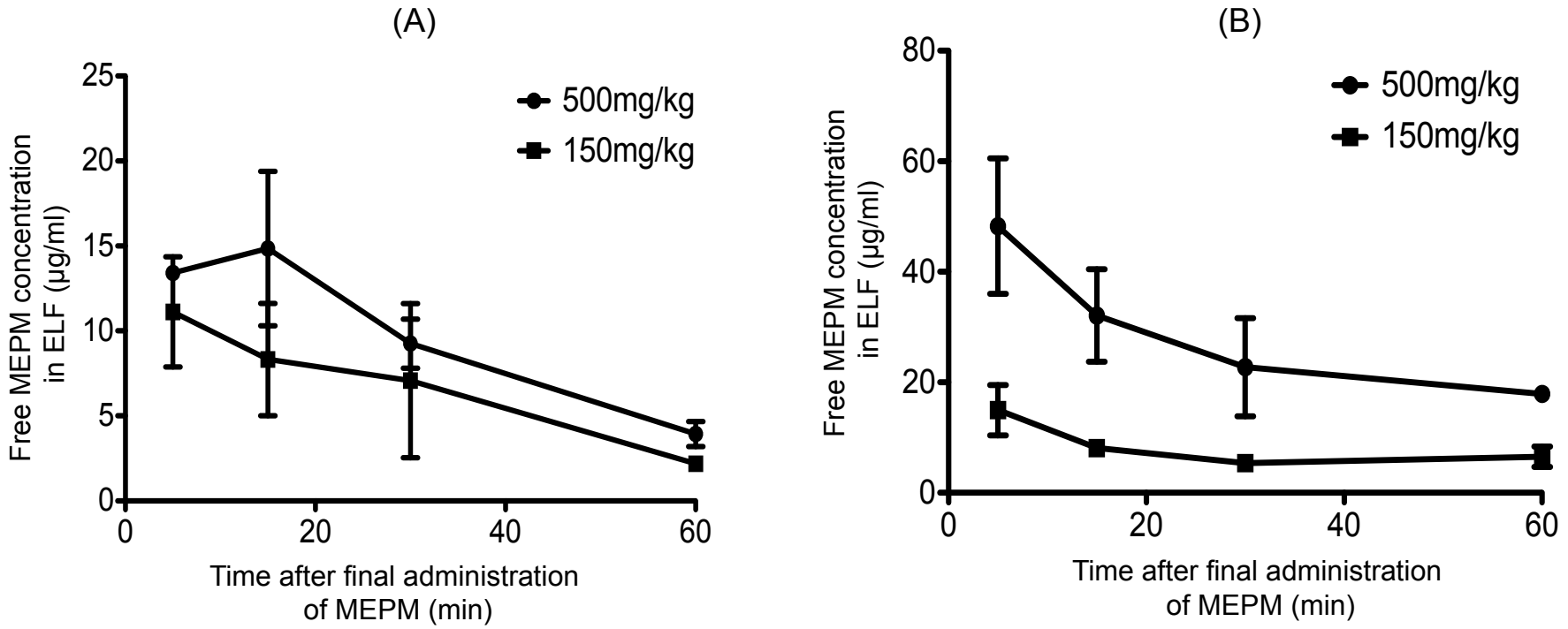


Figure 4. Effect of high-dose MEPM on histopathological analysis in lungs of mice inoculated with 3×10^6 CFU of MEPM-resistant *P.aeruginosa*. At 38 hours after infection (24 hour after 1st dose of MEPM), mice treated with MEPM at 500mg/kg × 4/day and 150mg/kg × 4/day and no treatment mice were compared. HE stained tissue sections were at magnifications of ×40 and ×200. No treatment group (A), 150mg/kg×4/day group (B), 500mg/kg×4/day group (C). The inflammation of lungs becomes mild by dose-dependent. The accumulation of inflammatory cells, hemorrhage in lungs and destruction of alveoli are limited in the mice treated with MEPM at 500mg/kg × 4/day.



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