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Comparative evaluation of intratracheal colistimethate sodium, imipenem, and meropenem in BALB/c mice with carbapenem-resistant *Acinetobacter baumannii* pneumonia

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SUMMARY

Objective: The identification of the optimal agent for administration via the respiratory tract when treating pneumonia caused by carbapenem-resistant *Acinetobacter baumannii* (CRAB).
Methods: A murine model of acute CRAB pneumonia was established by intratracheal (i.t.) inoculation with 2.5×10^7 colony-forming units (CFU) of *A. baumannii* strain Ab396 plus 10% porcine mucin. After 4 h the infected BALB/c mice were treated intratracheally with 25 μl of either 0.85% saline (control group), colistimethate sodium (CMS) (166 666 U/kg, CMS group), imipenem/cilastatin (30/30 mg/kg, imipenem group), or meropenem (20 mg/kg, meropenem group), every 8 h. The therapeutic efficacy of these agents was examined.
Results: A. baumannii strain Ab396 was susceptible to CMS only. However, meropenem treatment did give a significantly superior survival rate (100%) compared to treatment with imipenem (50%), CMS (33%), or saline (0%) ($p < 0.001$ vs. the control and CMS groups, $p = 0.006$ vs. the imipenem group, by log-rank test). Furthermore, compared to the other groups, the meropenem group demonstrated significantly more favorable results in terms of tissue penetration of the antibiotic, bacterial clearance, normalization of the wet lung-to-body weight ratio, and down-regulation of pro-inflammatory cytokine levels in the lungs.
Conclusions: Administration of meropenem via the respiratory tract proved to have the best therapeutic efficacy among the antibiotics tested when treating advanced murine CRAB pneumonia.

1. Introduction

Acinetobacter baumannii, a non-fermenting Gram-negative bacterium found in the natural environment,[1] is characterized by its ability to develop resistance to a majority of antimicrobials, and is an important nosocomial pathogen that is often involved in hospital outbreaks.[2,3] Patients with pneumonia due to carbapenem-resistant *A. baumannii* (CRAB) show a high mortality rate (30% to 75%) and require prolonged hospital stays in intensive care units (ICUs).[4,5] Notwithstanding the fact that carbapenems have been previously considered to be the gold standard drugs for severe *A. baumannii* infection,[6] nosocomial outbreaks caused by CRAB have been increasingly reported worldwide.[3,7] Handling such infections is an ongoing clinical challenge. At present, parenteral colistimethate sodium (CMS) therapy is used as a salvage therapy for multidrug-resistant (MDR) Gram-negative bacterial infections,[8,9] and leads to a favorable clinical response in 55% to 74% of CRAB-infected patients.[10–12] However, Levin et al. reported that parenteral CMS therapy achieved a good clinical response rate in only 25% of patients with MDR bacterial pneumonia.[13] Probably pertinent is the poor tissue penetration of CMS based on the findings of Montero et al., who reported that intraperitoneal CMS had limited therapeutic efficacy in CRAB pneumonia mice in comparison with imipenem or sulbactam.[14] As CRAB pneumonia

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has emerged in ICUs, the administration of CMS via the respiratory tract has become attractive as an adjunctive therapy for CRAB pneumonia patients,[15,16] due to the high local drug concentrations and low systemic toxicity.[17,18] In a comparative cohort study, the outcome of adjunctive inhaled CMS on ventilator-associated pneumonia (VAP) was better than parenteral CMS alone.[19]

Traditionally, systemically circulated antimicrobials have been used to treat pneumonia patients. However, administering antibiotics via the respiratory tract, an adjunctive therapy, is an interesting approach to treating pneumonia caused by MDR organisms because of the clinical threat of these bacteria.[20,21] A meta-analysis indicated that intratracheal (i.t.) or aerosolized antibiotic therapy may be useful when treating nosocomial pneumonia.[22] Additionally, the American Thoracic Society/Infectious Diseases Society of America guidelines state that aerosolized antibiotics combined with systemically administered antibiotics may have some advantages when treating some MDR pathogens[23] in VAP patients. Moreover, monotherapy with nebulized CMS may be an attractive option when treating MDR Gram-negative pneumonia in clinical practice[24] or in animal studies.[25] In addition, it has been shown that there is a better therapeutic outcome after treatment with i.t. colistin sulfate than when the drug is administered intraperitoneally; this was demonstrated in our murine model of early CRAB pneumonia, which involved analysis at 2 h after intratracheal inoculation with a CRAB isolate.[26]

However, penetration of antibiotics in the distal lungs may be hindered by bronchial plugs from the purulent secretions during advanced pneumonia,[25,27,28] and because of the poor tissue penetration of CMS, the topical administration of CMS may have only a limited therapeutic efficacy in advanced CRAB pneumonia. Moreover, the effectiveness of antibiotics administered via the respiratory tract for the treatment of CRAB pneumonia has not been extensively evaluated. Thus, it would be helpful to find an optimal antibiotic for treating advanced CRAB pneumonia via the respiratory tract. Parenteral carbapenems have been considered to be effective drugs when treating *A. baumannii* infections. Badia et al. reported that intratracheal administration of imipenem was able to achieve high local drug concentrations and thus has potential when treating VAP caused by MDR Gram-negative bacteria.[29] Hence, like CMS, the administration of carbapenems via the respiratory tract might be a potential therapy for CRAB pneumonia. Therefore, in this study we compared the therapeutic efficacy of i.t. CMS, imipenem/cilastatin, and meropenem when treating CRAB pneumonia (4 h after intratracheal inoculation with a CRAB isolate) in mice.

2. Materials and methods

2.1. Microorganism and antibiotics

A. baumannii strain Ab396 was isolated from the bloodstream of a clinical patient, and identified by the Phoenix 100 (Baxter Health Care, USA). All aliquots were prepared and frozen at −80 °C until used. Standard CMS powder was obtained from TTY Biopharm (Taoyuan, Taiwan). Colistin sulfate was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Imipenem was obtained from Merck (Virginia, USA). Meropenem was supplied by Dainippon Sumitomo, Osaka, Japan.

2.2. Determination of minimum inhibitory concentrations (MICs)

MIC values for colistin sulfate, meropenem, and imipenem for *A. baumannii* strain Ab396 were determined by the agar dilution method, and MIC interpretive breakpoints were identified following the recommendations of the Clinical and Laboratory Standards Institute.[30]

2.3. Mice

Six- to eight-week-old inbred BALB/c female mice weighing 20–22 g, purchased from the Animal Center of the National Science Council, Taipei, Taiwan, were allowed to equilibrate for 5–7 days at the Animal Center of Chi Mei Medical Center. Food and water were supplied ad libitum, light was controlled to provide 12-h day-and-night periods, and the room air was HEPA filter-purified.

2.4. Mouse model for acute pneumonia

The acute pneumonia model was established in mice, as previously described.[26] In brief, the mice were anesthetized with sodium pentobarbital at a dose of 70 mg/kg, and were supported in a frame. The airway was illuminated externally by a lamp and the vocal folds were exposed. A micropipette was passed down the pharynx, and its tip was inserted between the vocal folds. At this point 50 μl of a bacterial suspension containing 2.5×10^7 colony-forming units (CFU) of bacteria and 10% porcine mucin was instilled. The animal was maintained in an upright position for 2 min to allow the suspension to drain into the respiratory trees. Based on the pulmonary pathology results of our previous study,[26] in which acute pneumonia developed at 4 h after bacterial instillation, treatment with the antimicrobial agents was initiated at this time point. All animal experiments were approved by the Chi Mei Medical Center Animal Center. Four cohorts of mice, namely the control, CMS, imipenem, and meropenem groups, were used during the following experiments.

2.5. Survival study

Since no dosing regimens with respect to an appropriate i.t. dose for mice are available, the i.t. dosages chosen followed the parenteral dosages as previously described.[14,31,32] For each group, 12 infected mice at 4 h after i.t. *A. baumannii* strain Ab396 inoculation were treated i.t. with 25 μl of either 0.85% saline (control group), or imipenem/cilastatin (30/30 mg/kg, imipenem group), or CMS (166 666 U/kg, CMS group), or meropenem (20 mg/kg, meropenem group), every 8 h for 48 h. The survival rates were recorded every 12 h until 72 h after i.t. *A. baumannii* strain Ab396 inoculation.

2.6. Pulmonary bacterial clearance, cytokines, and wet lung-to-body weight ratios

The mice with CRAB pneumonia were treated with one of three drugs or saline, as in the above survival study. After treatments began, eight surviving mice that were tested at 24 h, 48 h ($n = 8$ for each group), and 72 h ($n = 8$ for the imipenem, CMS, and meropenem groups) were identified and sacrificed by an overdose of intraperitoneal pentobarbital. After being weighed, homogenized lung samples were examined to measure the pulmonary bacterial load. Ten-fold serial dilutions of the homogenized lungs were resuspended in saline, and 0.1 ml of each dilution was spread on agar plates. The bacterial colonies growing in the plates were counted and the results expressed as $\log_{10}$ CFU/mg of homogenized lung at 24, 48, and 72 h. The lower limit of detection was 1.5 $\log_{10}$ CFU/mg. The lung samples were also examined for the wet lung-to-body weight ratio at 24, 48, and 72 h.

In order to measure the cytokine levels in bronchoalveolar lavage fluid (BALF), eight surviving mice at 24 h, 48 h ($n = 8$ for each group), and 72 h ($n = 8$ for the imipenem, CMS, and meropenem groups) were separated from each group and sacrificed with an intraperitoneal dose of 70 mg/kg of pentobarbital. The trachea of each infected mouse was cannulated with a 25-gauge needle and 1 ml of 0.85% saline was infused into the lungs via the needle. After this process, about 0.95 ml of BALF specimen was obtained by