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Note

Colistin loading dose enhanced antimicrobial activity for *in vivo* mouse thigh infection model with *Pseudomonas aeruginosa* with highly antimicrobial resistant

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ABSTRACT

This is the first report to test the loading dosage of colistin against *Pseudomonas aeruginosa*, including MDRP. Using *in vivo* murine thigh infection model, in the loading dosage regimen (Day 1:50 mg/kg q12 h, Day 2–3: 25 mg/kg q12 h) group, 5 to 6 log10 CFU/ml reduction compared with control were observed for both strains of *P. aeruginosa* with colistin MIC 0.5 and 1 μg/mL at 72 h. But, similar reduction was observed for the strains with colistin MIC 0.5 μg/mL only in normal dosage regimen (Day 1–3: 25 mg/kg q12 h) group. For *P. aeruginosa* with colistin MIC 1 μg/mL, colistin loading dosage regimens showed greater antimicrobial activity than that of without loading dosage group ($p < 0.05$). These data suggest that the colistin loading regimen would be one of the useful options for *P. aeruginosa* with antimicrobial resistance infection treatment.

1. Introduction

Pseudomonas aeruginosa is a clinical significant Gram-negative rod. And *P. aeruginosa* with antimicrobial resistant, especially for multidrug resistant *P. aeruginosa* (MDRP), have emerged and threatening the utility of a mainstay in anti-pseudomonal therapy [1–4]. Then, the limited antimicrobial choice make more difficult to treat the infections. Herein, it is important to explore optimal antimicrobial therapy against *P. aeruginosa*, including MDRP.

The blood colistin concentration took 2 days to reach steady state. The fact suggested the benefits of treatment initiation with a loading dose [5]. The implications of pharmacokinetic findings are that the plasma colistin concentrations are insufficient before steady state and raise the question of whether the administration of a loading dose would benefit for ill patients. But, they recommended the loading dose of colistin only based on the pharmacokinetic data. Few previous studies have revealed its antibacterial or clinical benefits since clinical studies could be affected by several factors [6–8]. Thus, we evaluated the antimicrobial efficacy of the colistin loading regimen against *P. aeruginosa*, including MDRP, with *in vivo* mouse thigh infection model.

Commercial available colistin (JHP Pharmaceuticals, LLC., Rochester, MI) was used for *in vivo* study. Immediately before experimentation, colistin was reconstituted and diluted with normal saline to achieve the desired concentration. *P. aeruginosa* strains were isolated at Aichi Medical University Hospital, Aichi, Japan. For *in vivo* study, we used three *P. aeruginosa* strains, including 2 MDRP strains. The study was reviewed and approved by the Aichi Medical University Hospital Institutional Animal Care and Use Committee.

We determinated MIC of antimicrobials with E-test or the microdilution method according to the manufacture's specifications (bioMérieux North America, Durham, NC) or the Clinical and Laboratory Standards Institute (CLSI) guidelines. MDRP strains resistant to the following three antimicrobial groups: carbapenems (MIC of imipenem ≥16 μg/mL), aminoglycosides (MIC of amikacin ≥32 μg/mL) and fluoroquinolones (MIC of ciprofloxacin ≥4 μg/mL).

Pathogen-free female ICR mice weighing about 22 g were purchased from Charles River Laboratories Japan Inc. (Yokohama, Japan), and utilized throughout experiments. The mouse thigh

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infection model described by Hagihara et al. [9,10] was adopted to examine the relationship between colistin exposure and reduction in the density of *P. aeruginosa* in the thigh muscles of neutropenic mice. The efficacy was evaluated as the change in bacterial density in the thigh muscles.

At first, the following doses of colistin were evaluated as dose–response study: 0 (control), 5, 10, 20, 40, 50 and 80 mg/kg q12 h. Thighs from the animals were harvested 24 h. The doses selected for the dose–response study were predicted to fall on the steep part of the sigmoid *Emax* dose–response to ease detection of changes in response. The indices *Emax* and ED_{50} were estimated by nonlinear least-squares regression (WinNonlin software ver. 6.3., Pharsight Inc.).

Based on the results of the dose–response study, $2 \times ED_{75}$ and ED_{75} dose used as loading dose and maintenance dose of colistin in pharmacodynamic study. Some previous studies showed that clinical cure rate of colistin with normal dosage regimen 1.25–2.5 mg/kg q12 h was around 70–80% for various patients [11]. Thighs from all the animals were harvested 72 h after the initiation of therapy.

The significance of differences in bacterial densities between groups was evaluated by analysis of variance. A difference was considered significant at a p value of <0.05. To compare antimicrobial efficacy between regimens, oneway ANOVA was used.

The antimicrobial susceptibilities of imipenem/cilastatin, amikacin, ciprofloxacin, colistin. were >32, 64, >32, 1 μg/mL for AMU 13-4632, 8, 64, >32, 0.5 μg/mL for AMU 14-1860, >32, 64, >32, 0.5 μg/mL for AMU 14-6735, we used in this study.

The results of the dose–response studies are shown in Fig. 1. During the studies, the 0 h control mice displayed a mean (±standard deviation) bacterial density of 5.40 ± 0.37 $\log_{10}$ CFU/ml. The colistin therapy at 5–80 mg/kg q12 h doses resulted in reductions in bacterial density of −2.22 to 3.79 $\log_{10}$ CFU/ml after 24 h inoculation. This study demonstrated marked bactericidal activity that was dose dependent. The maximal effect was observed with colistin doses of ≥50 mg/kg q12 h. The relationship of the $\Delta\log_{10}$ CFU/ml with colistin dosage, colistin monotherapy produced reductions in bacterial density after 24 h against the *P. aeruginosa* strain (AMU 14-6735) tested ($R^2 = 0.95$). The ED_{50} was 25 mg/kg q12 h. Based on the result, we selected 50 mg/kg q12 h as loading dose and 25 mg/kg q12 h as maintenance dose for the loading dosage group (Day1: 50 mg/kg q12 h, Day2–3: 25 mg/kg q12 h) and for the normal dosage regimen group (Day 1–3: 25 mg/kg q12 h).

The results of the pharmacodynamic studies are shown in Fig. 2. Three strains grew to 7.65–8.58 $\log_{10}$ CFU/ml after 72 h in untreated control animals. Colistin monotherapy with loading dose achieved greater $\log_{10}$ CFU/ml reductions than that of the normal dosage regimen against *P. aeruginosa* strains (AUM13-4632) with colistin MIC 1 μg/mL 72 h after colistin therapy started (−0.72 ± 0.25 vs −3.13 ± 0.26; $p < 0.05$) (Fig. 2). The antimicrobial activity in loading dosage group was not affected with antimicrobial susceptibility within colistin MIC 0.5–1 μg/mL (Fig. 2). On the other hands, AUM13-4632 with colistin MIC 1 μg/mL showed minimum bacterial reduction, compared with the other *P. aeruginosa* with colistin MIC 0.5 μg/mL (AUM13-4632 and AUM13-4632) at 72 h (−0.72 ± 0.25 vs −2.62 ± 0.43 and −2.17 ± 0.54; $p < 0.05$).

MDRP infections have few effective drugs. Colistin is still a key antimicrobial agent used as a therapy [4]. Colistin is generally used as 1.25–2.5 mg/kg q12 h for infectious treatment. Some experts recommended to use loading dose as 2*maintenance dose especially for sever infected patients. Hence, our study used higher dosage regimens as maintenance dose and loading dose to simulate antimicrobial activity of colistin previous clinical study revealed [11,12]. In our study, the colistin dosage was selected based on the dose–response study results. Some previous studies showed that clinical cure rate of colistin with normal dosage regimen was about 70–80% for various patients. Thus, we selected the dose showed ED_{75} (25 mg/kg q12 h) and $2*ED_{75}$ (50 mg/kg q12 h) as maintenance dose and loading dosage.

When the initial dose was doubled, the unbound colistin concentrations were more than doubled. The facts lead to an even more efficient bacterial kill from higher doses [13]. The pharmacokinetic model and the semi-mechanistic pharmacokintic/pharmacodynamic model developed in previous study predicted that a loading dose can indeed be of importance for rapid clearance of bacteria. Because, colistin concentrations increase slow when the

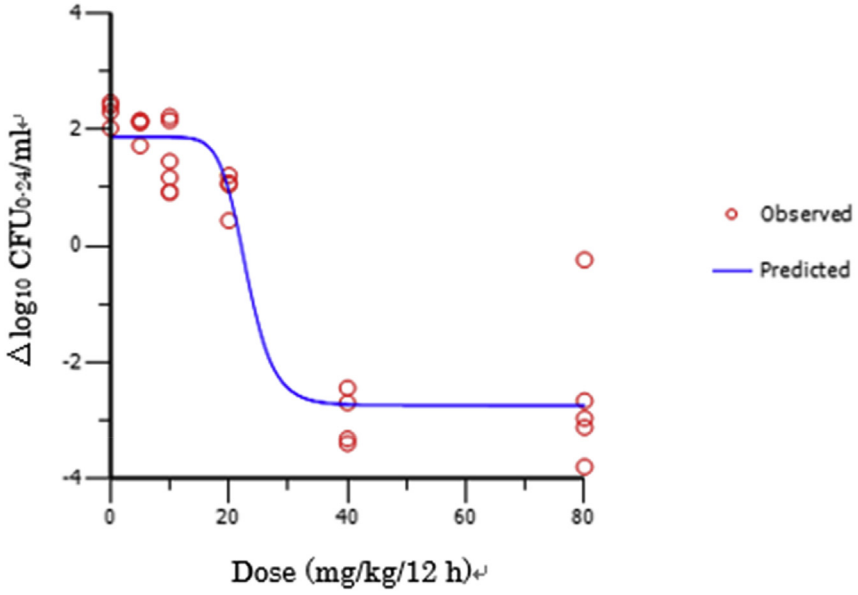

Fig. 1. Dose-response study of colistin against *P. aeruginosa* strain (AMU 14-6735). Change in $\log_{10}$ CFU_{0-24}/ml per thigh for colistin monotherapy with various doses at 24 h, relative to that for 0-h controls for a collection of *P. aeruginosa* isolates tested in neutropenic studies.