

Efficacy of doripenem in the treatment of *Pseudomonas aeruginosa* experimental pneumonia versus imipenem and meropenem

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Objectives: The aim of this study was to compare doripenem with imipenem and meropenem in an experimental rabbit model of *Pseudomonas aeruginosa* pneumonia and then to compare different doripenem doses and methods of intravenous administration.

Methods: Using a rabbit experimental model of pneumonia, efficacy was assessed following 2 days of treatment by colony counts of different tissues (lung, spleen and blood culture).

Results: Mean pulmonary bacterial loads were 3.17 ± 0.53 , 3.42 ± 0.61 and 2.75 ± 0.59 log₁₀ cfu/g for imipenem, doripenem (0.5 g three times daily) and meropenem, respectively, compared with 7.57 ± 0.99 cfu/g for control animals. At a higher dose (1 g three times daily), doripenem showed significantly better efficacy (2.70 ± 0.65 log₁₀ cfu/g) than the standard regimen of doripenem. Sterilization of spleen cultures was achieved with standard regimens of imipenem (1 g three times daily) and a higher dose of doripenem.

Conclusions: In this model of *P. aeruginosa* pneumonia, doripenem had an efficacy equivalent to that of meropenem and imipenem at a high dose of 1 g three times a day and lower efficacy at a standard dose (0.5 g three times daily) than the other two agents in terms of bacteria cultivated from spleens. Doripenem is a new drug that offers new therapeutic options, especially for difficult-to-treat infections such as pneumonia due to non-fermenting Gram-negative bacteria.

Keywords: animals, β -lactams, administration, dosage, metabolism, therapeutic use, pharmacokinetics

Introduction

Pseudomonas aeruginosa is one of the most common Gram-negative organisms associated with nosocomial infections, particularly in immunocompromised and critically ill patients. It is responsible for a broad range of clinical manifestations, including respiratory tract infections, intravascular device-associated infections, urinary tract infections and post-operative infections. The increasing frequency of multidrug-resistant strains is of great concern as efficacious antimicrobial options are severely limited.^{1–3}

Doripenem is a new 1- β -methylcarbapenem with a low MIC for *P. aeruginosa*. The drug has been approved for use in nosocomial pneumonia as well as in complicated abdominal and urinary tract infections. However, a comparison with other carbapenems has not yet been performed *in vivo*.

The aim of this study was to compare doripenem with imipenem and meropenem in an experimental rabbit model of

P. aeruginosa pneumonia and then to compare different doripenem doses and methods of intravenous administration.

Materials and methods

Antimicrobial drugs

Clinical forms of the following antibiotics were used for the experiments: doripenem (Janssen-Cilag, Issy-les-Moulineaux, France); imipenem/cilastatin (Merck Sharp & Dohme-Chibret, Paris, France); and meropenem (AstraZeneca, Rueil-Malmaison, France). The drugs were dissolved in saline just before administration, according to the manufacturers' recommendations.

Bacterial strain

P. aeruginosa PAO1 was used as the infective strain. Bacteria were cultured on Mueller–Hinton agar plates (bioMérieux, Marcy l'Étoile, France) at 37°C. Drug MICs were determined by standard agar dilution

as recommended by the Comité de l'antibiogramme de la Société Française de Microbiologie.⁴

The experimental protocol was approved by the Committee of Animal Ethics of the University of Nantes. Animals were treated in accordance with national regulations on animal regulation.

Experimental *P. aeruginosa* pneumonia in rabbits

Female New Zealand White immunocompetent rabbits (body weight 2.5–3.2 kg) were obtained from CEGAV (Saint Mars d'Egrenne, France) and placed in individual cages. Food and water were provided *ad libitum*.

Venous and arterial catheters were inserted and pneumonia was initiated as described previously.^{5–7} In brief, bacterial pneumonia was induced by endobronchial challenge with 1 mL of saline containing *P. aeruginosa* at a final concentration of 9.5 log₁₀ cfu/mL. Antibiotic treatment was started 5 h after bacterial challenge and lasted 2 days. Antibiotics were delivered through a venous catheter, with changing infusion rates delivered by a computer-controlled electric pump, in order to simulate the pharmacokinetics observed in human serum.^{8,9}

Animals were randomly assigned to six groups: (i) no treatment (controls); (ii) discontinuous imipenem group [computer-controlled syringe pump infusion simulating a human equivalent (HE) dose of 1 g three times daily]; (iii and iv) discontinuous doripenem groups (simulating either an HE dose of 0.5 g three times daily or an HE dose of 1 g three times daily); (v) continuous doripenem group (continuous infusion of doripenem at a dose reproducing an AUC equivalent to 1.5 g a day); and (vi) discontinuous meropenem group (simulating an HE dose of 1 g three times daily). These simulations reflect typical recommended clinical doses.^{10,11} Eight animals were randomly assigned to each treatment group and 10 animals were assigned to the control group. Antibiotic infusions were stopped 4 h before the animals were euthanized.

Pharmacokinetic analysis

For each animal, plasma antibiotic concentrations were determined in blood samples obtained through an arterial catheter. Antibiotic concentrations were determined by microbioassay using antibiotic medium II (Difco) and *Bacillus subtilis* ATCC 9466 as the indicator organism (sensitivity threshold of 0.1 mg/L). Standard curves were established with antibiotic solutions in plasma. The linearity of these curves was ≥ 0.98 (r^2). If necessary, plasma samples were diluted to ensure that concentrations were within the range of those on the standard curve. Standards were assayed for each experiment and plasma concentrations were determined in duplicate.

Evaluation of infection

At the end of the treatment (2 days), animals were euthanized following international guidelines. The spleen and both lungs from each animal

were weighed and homogenized in 1 mL of saline buffer and used for quantitative cultures on agar for 24 h at 37°C.

Dilutions at 10⁻¹, 10⁻² and 10⁻⁴ were performed to eliminate potential carry-over effects. Viable counts, after 24 h of incubation, were expressed as the mean \pm SD log₁₀ cfu per g of lung.

Blood cultures were also performed. Spleen and blood culture results were expressed qualitatively (positive or negative).

To evaluate whether doripenem, imipenem and meropenem treatment could induce the selection of *in vivo* resistant variants, undiluted vegetation homogenates were spread on agar plates containing the appropriate antibiotic at a concentration corresponding to 4-fold the MIC. Bacterial counts were determined after 48 h of incubation at 37°C.

Spontaneous mortality was 20% (2 out of 10 in the control group).

Statistical analysis

Statistical analyses were performed using Microsoft® Office Excel 2003, SPSS® 13.0 and Graphpad Prism® 4 for Windows (Graphpad Software, San Diego, CA, USA). Results were expressed as means \pm SD. Quantitative variables were compared using one-way ANOVA. This analysis was completed with a *post hoc* Bonferroni test. Proportions (percentages) were compared using Fisher's exact test. $P < 0.05$ was considered statistically significant.

Three treatment groups were compared: imipenem; doripenem (0.5 g three times daily); and meropenem. Different dosage regimens for doripenem (0.5 g, 1 g and continuous infusion) were then analysed.

Results

MICs

The MICs for *P. aeruginosa* PAO1 were 2 mg/L for imipenem and meropenem and 1 mg/L for doripenem.

Pharmacokinetics

Pharmacokinetic/pharmacodynamic parameters are summarized in Table 1. The actual exposure curves are presented in Figures S1 to S4 [available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>)].

Comparison of the efficacy of doripenem, imipenem and meropenem

Two of the 10 control animals died before evaluation. This difference was not statistically significant.

No resistant mutant was isolated among the surviving bacteria from any treated animal. Mean pulmonary bacterial loads

Table 1. Pharmacokinetic/pharmacodynamic parameters for the different treatment groups (imipenem, meropenem and doripenem)

	Imipenem 1 g three times daily	Meropenem 1 g three times daily	Doripenem 0.5 g three times daily	Doripenem 1 g three times daily	Doripenem 1.5 g a day (continuous infusion)
C_{max} , mean \pm SD (mg/L)	91 \pm 16	57 \pm 10	52 \pm 11	73 \pm 8	13 \pm 3
$t_{1/2}$ (h)	1.18	1.38	1.72	1 ^a	NA
Percentage of time above MIC	88	88	100	100	100
Estimated AUC (mg·h/L)	76	53	89	102 ^a	78
AUC/MIC	38	26.5	89	102 ^a	78

NA, not available.

^aThese parameters must be interpreted with caution because of the low number of available data in this group (<50% of the animals).

Table 2. Pulmonary bacterial load and spleen and blood culture results for the different treatment groups (imipenem, meropenem and doripenem) and controls

	Imipenem 1 g three times daily	Doripenem 0.5 g three times daily	Meropenem 1 g three times daily	Controls	<i>P</i> ^a
Pulmonary bacterial load ^b , mean ± SD (log ₁₀ cfu/g)	3.17 ± 0.53	3.42 ± 0.61	2.75 ± 0.59	7.57 ± 0.99	<10 ⁻⁴
Spleen cultures positive/negative	1/7	6/2	4/4	6/2	0.04
Blood cultures positive/negative	1/7	0/8	1/7	2/6	0.886

^aQuantitative variables were compared using one-way ANOVA. Proportions (percentages) were compared using Fisher's exact test.

^bBacterial load did not differ significantly when the antibiotics were compared using a *post hoc* Bonferroni test.

Table 3. Pulmonary bacterial load and spleen and blood culture results for the different doripenem groups

	Doripenem 0.5 g three times daily	Doripenem 1 g three times daily	Doripenem 1.5 g a day (continuous infusion)	<i>P</i> ^a
Pulmonary bacterial load ^b , mean ± SD (log ₁₀ cfu/g)	3.42 ± 0.61	2.70 ± 0.65	3.90 ± 0.81	<10 ⁻²
Spleen cultures positive/negative	6/2	0/8	7/1	<10 ⁻³
Blood cultures positive/negative	0/8	0/8	3/5	0.083

^aQuantitative variables were compared using one-way ANOVA. Proportions (percentages) were compared using Fisher's exact test.

^bA *post hoc* Bonferroni test showed a statistically significant difference between the doripenem 1 g three times daily and doripenem 1.5 g groups.

were statistically different (treatment groups versus controls; *P* < 0.0001). However, pulmonary bacterial load did not differ significantly when the three antibiotic treatments were compared (Table 2).

The results of spleen cultures are also shown in Table 2. Treatment with imipenem and meropenem resulted in significantly more culture-negative spleen cultures than doripenem at a dosage of 0.5 g three times daily (*P* < 0.05). Conversely, there was no significant difference in blood culture results between the three groups (Table 2). Results for bacterial pulmonary load are also shown in Figures S5 to S7 [available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>)].

When comparing the imipenem and meropenem groups with doripenem at a dosage of 1 g three times daily, no difference was noted either for pulmonary bacterial load or for spleen and blood cultures.

Comparison of the different doripenem regimens

Mean pulmonary bacterial loads were significantly different between these three groups (Table 3). *Post hoc* analysis showed a statistical difference between doripenem 1 g three times daily and doripenem 1.5 g a day (*P* < 0.01).

Table 3 also shows the results of spleen cultures for the different doripenem groups. Spleen cultures were more often negative in the doripenem 1 g three times daily group compared with doripenem 0.5 g three times daily and doripenem 1.5 g a day (*P* < 0.001). However, there was no difference in blood culture results between the three groups.

Discussion

This study provides efficacy data for doripenem compared with the reference carbapenems imipenem and meropenem in an experimental model of *P. aeruginosa* pneumonia.

Doripenem is a new drug that offers new therapeutic options, especially for difficult-to-treat infections such as pneumonia due to non-fermenting Gram-negative bacteria. Its activity *in vitro* is comparable to meropenem, but it is more efficacious than imipenem/cilastatin against *P. aeruginosa*.¹² On the other hand, meropenem is often considered more active *in vitro* against *Acinetobacter baumannii* strains than imipenem or doripenem.^{13,14} The pharmacokinetic profile of doripenem is very similar to that of imipenem/cilastatin and meropenem.¹⁵ Its 1-β-methyl side chain provides some resistance to dehydropeptidase, so that this molecule does not require the addition of cilastatin for protection from this enzyme.¹⁶ It is also remarkably stable after reconstitution (12 h after reconstitution in 0.9% NaCl and 4 h in 5% dextrose), increasing the opportunity for prolonged infusion and the potential to extend the pharmacodynamic parameter *fT*_{>MIC} (time that free plasma drug concentration exceeds the MIC for the infecting pathogen).¹⁷ The recommended dosing for doripenem is 0.5 g every 8 h (administered via 1 h or 4 h infusions).

Controlled clinical trials have been carried out comparing doripenem with meropenem in complicated intra-abdominal infections¹⁸ and with imipenem in ventilator-associated pneumonia.¹¹ Non-inferiority of doripenem was reported in both of these studies.

Our purpose in this study was to compare two doripenem doses (the recommended regimen of 0.5 g three times daily versus a high-dose regimen of 1 g three times daily) and two infusion modalities (discontinuous versus continuous) in an experimental model of pneumonia. This experimental model of *P. aeruginosa* pneumonia is of interest because it allows simulation of human pharmacokinetics; *C*_{max}, *t*_{1/2} and AUC were all consistent with available human data.^{19,20} The reproducibility of this model was good; all animals had positive lung cultures and 75% had positive spleen cultures. The severity of this

experimental infection was also attested by the mortality rate (20% among non-treated animals).

In this model, a standard doripenem dose of 0.5 g three times a day exhibited efficacy similar to the reference carbapenems imipenem and meropenem. Nevertheless, sterilization of spleen cultures (often considered a good reflection of systemic infection) was achieved more effectively with standard regimens of imipenem (1 g three times daily) and meropenem (1 g three times daily) than with doripenem at a dose of 0.5 g three times a day. However, increasing the doripenem dose from 0.5 g to 1 g three times daily led to a significantly higher reduction in pulmonary bacterial load and a higher rate of spleen sterilization. No difference was observed for blood cultures since sterilization was already obtained with the standard doripenem dose.

For carbapenem antibiotics, the fraction of time during the dosing interval that the drug concentration remains above its MIC for the infecting pathogen(s) ($fT_{>MIC}$) is considered the target that best relates (directly) to patient outcomes.²¹ Nevertheless, in this work, $fT_{>MIC}$ was high in each group and cannot explain the differences observed between the doripenem groups since the study was not designed for this purpose.

These results confirm the high efficacy of doripenem at its recommended dose, in comparison with imipenem/cilastatin and meropenem. Nevertheless, sterilization of spleen cultures was more effective with the higher-dose regimen. Since spleen cultures reflect systemic diffusion of infection due to bacteraemia, this suggests that the high-dose regimen would be better to treat more severe infections.

In this experimental model of *P. aeruginosa* pneumonia, doripenem at a dose of 0.5 g three times a day had equivalent efficacy to that of other carbapenems (meropenem and imipenem) in terms of pulmonary bacterial load, but lower efficacy in terms of number of spleens with viable bacteria. However, at a higher dose (1 g three times daily) doripenem showed significantly better efficacy (greater reduction in pulmonary bacterial load and higher rate of spleen sterilization) than other doripenem dosing regimens.

The data are of interest to clinicians treating severely ill patients, such as those on intensive care units. For these patients, discontinuous administration of high doses (HE of 1 g three times a day) seems to be more efficient than conventional dosing (either discontinuous or continuous intravenous) and should be recommended.

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Transparency declarations

None to declare.

Supplementary data

Figures S1 to S7 are available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

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