

Efficacy of doripenem against *Escherichia coli* and *Klebsiella pneumoniae* in experimental meningitis

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Objectives: In this study the efficacy of doripenem, a new broad-spectrum carbapenem, was tested against an *Escherichia coli* strain and a *Klebsiella pneumoniae* strain in an experimental animal model. The comparator was cefepime monotherapy.

Methods: The rabbit meningitis model was used in this study and the penetration of doripenem through uninflamed and inflamed meninges was determined.

Results: Doripenem, injected three times (75 mg/kg), led to serum peak levels around 100 mg/L and trough levels around 5 mg/L, resulting in a penetration rate of 14% through inflamed meninges and 7% through uninflamed meninges. Against *K. pneumoniae*, doripenem was slightly but not significantly more efficacious than cefepime over 8 h ($5.40 \pm 1.37 \log_{10}$ cfu/mL versus $3.59 \pm 0.89 \log_{10}$ cfu/mL for cefepime). Also against the *E. coli* strain doripenem was slightly superior to the comparator ($5.55 \pm 0.87 \log_{10}$ cfu/mL versus $3.80 \pm 1.10 \log_{10}$ cfu/mL for cefepime), although the difference was not significant.

Conclusions: Doripenem is a potential monotherapy for the treatment of meningitis due to Gram-negative microorganisms.

Keywords: carbapenems, kinetics, rabbit meningitis

Introduction

The treatment of serious infections has been increasingly compromised by the continuous emergence of Gram-negative and Gram-positive resistant nosocomial pathogens.^{1,2} Antibiotics sharing the carbapenem ring are attractive candidates in the empirical treatment of nosocomial infections because of their broad antimicrobial spectrum. In general, carbapenems harbour a high efficacy against the major Gram-negative and Gram-positive pathogens, except against *Enterococcus faecium* and oxacillin-resistant staphylococci.^{3–5}

Doripenem is a new 1- β -methyl-carbapenem with a specific side chain substitution increasing its activity against non-fermentative Gram-negative microorganisms.⁶ In addition, doripenem is not hydrolysed by renal dehydropeptidases and stable to common β -lactamases, including extended-spectrum β -lactamases (ESBLs).⁷

Doripenem has been widely used in the treatment of nosocomial infections. It has been approved by the European Medicines Agency for the treatment of nosocomial pneumonia including ventilator-associated pneumonia, complicated intra-abdominal infections and complicated urinary tract infections including pyelonephritis. In the USA it has been approved by the US FDA for complicated intra-abdominal infections and complicated urinary tract infections including pyelonephritis.⁸

However, little is known about the penetration of doripenem into the CSF. In the experimental rabbit meningitis model, we have already determined the penetration of two other commonly used carbapenems, i.e. meropenem and ertapenem, and we have tested their efficacy in meningitis due to penicillin-resistant pneumococci.^{9,10}

The aim of this study was to test the efficacy of doripenem in experimental meningitis against two Gram-negative pathogens,

i.e. *Klebsiella pneumoniae* and *Escherichia coli*, and to determine its penetration through inflamed and uninflamed meninges.

Materials and methods

Strains and antibiotics

K. pneumoniae (1173687) and *E. coli* (QK-9) were kindly provided by the Institute for Infectious Diseases, University of Bern. Both strains were approved by the Kantonales Veterinäramt des Kantons Bern. Pathogen-free New Zealand rabbits were provided by the Zentraltierställe der Medizinischen Fakultät der Universität Bern, where all the experiments were performed.

Experimental meningitis model

The experimental rabbit meningitis model described by Dacey and Sande¹¹ was used in this project. The experimental protocols were approved by the Kantonales Veterinäramt des Kantons Bern. Pathogen-free New Zealand rabbits were provided by the Zentraltierställe der Medizinischen Fakultät der Universität Bern, where all the experiments were performed.

One day before an experiment, rabbits were anaesthetized with intramuscular injections of ketamine (30 mg/kg) and xylazine (15 mg/kg), and fitted with prostheses on their calvarium to facilitate subsequent placement within a stereotactic frame. On the day of the experiment, rabbits received 1.75 g/kg ethylcarbamate (urethane) by subcutaneous injection and then 10 mg/kg pentobarbital by intravenous (iv) injection to induce deep anaesthesia. The animals were fixed in a stereotactic frame, and a 3.5 inch (25G) spinal needle was introduced into the cisterna magna. Following the withdrawal of 0.2 mL of CSF, the test bacteria (1×10^5 cfu in 0.2 mL of saline solution) were injected into the subarachnoid space. After inoculation the animals were brought back to their cages for the night. The next day the rabbits were again fitted in the frames using the techniques and anaesthesia described above. A catheter was fixed in the femoral artery for serum sampling. A spinal needle was fixed again in the subarachnoid space. Doripenem was injected (75 mg/kg iv) at hours 0, 3 and 6 because of its short half-life in rabbits. The comparator regimen, cefepime (100 mg/kg), was injected at hours 0 and 4, as previously described.^{12,13}

CSF (0.2 mL) was sampled at 0, 1, 2, 4, 6 and 8 h after infection. Blood was sampled at 0.25, 0.5, 1, 2, 3, 4, 4.25, 4.5, 5, 6, 7 and 8 h. Each experimental series included untreated control rabbits. In each group 10 rabbits were treated.

Determination of antibiotic levels and cfu titres

The doripenem concentration in serum and CSF, and the cefepime concentration in the CSF were determined by diffusion microbioassays using agar plates containing *Bacillus subtilis* (sus-1-A), 10^6 cfu/0.1 mL (Raven Biological Laboratories, Inc.).^{14,15}

cfu were determined by serial dilution of CSF. Samples thereof (20 μ L) were plated on agar plates and incubated overnight at 37°C. The limit of detection of this assay was therefore 50 cfu/mL ($1.7 \log_{10}$ /mL).

The penetration into the CSF was determined by comparing areas under the curves (AUCs) for serum and CSF using GraphPad Prism Software, version 5.0 (GraphPad Software Inc., San Diego, CA, USA).

Statistical analysis

Student's *t*-test and one-way analysis of variance (Tukey–Cramer multiple comparisons test) were used for parametric data. Comparison of positive and negative cultures was analysed by the two-tailed Fisher exact test (GraphPad Prism Software, version 5.0). A *P* value <0.05 was considered significant.

The efficacy of the different regimens is presented as $\Delta \log_{10}$ cfu/mL/h and as $\Delta \log_{10}$ cfu/mL/8 h.

A value of $1.7 \log_{10}$ cfu/mL was assigned to the first sterile CSF sample and a value of 0 to each following sterile CSF sample.

Results

The kinetic properties of doripenem are presented in Figure 1(a and b). Due to its short half-life, doripenem had to be injected three times (3×75 mg/kg). At 15 min after the first injection, doripenem peaked at about 100 mg/L in the serum, but decreased rapidly to 2 mg/L 2 h later. The second injection led to similar peak levels that decreased again before the third injection. The effect of the third injection was comparable. In the inflamed CSF, the levels increased progressively after the first injection, with peak levels around 10 mg/L, decreasing to 2 mg/L before the next injection. After the second injection, CSF levels increased again to around 10 mg/L and decreased again by the same order of magnitude. At the end of the experimental period, the CSF levels were about 7 mg/L.

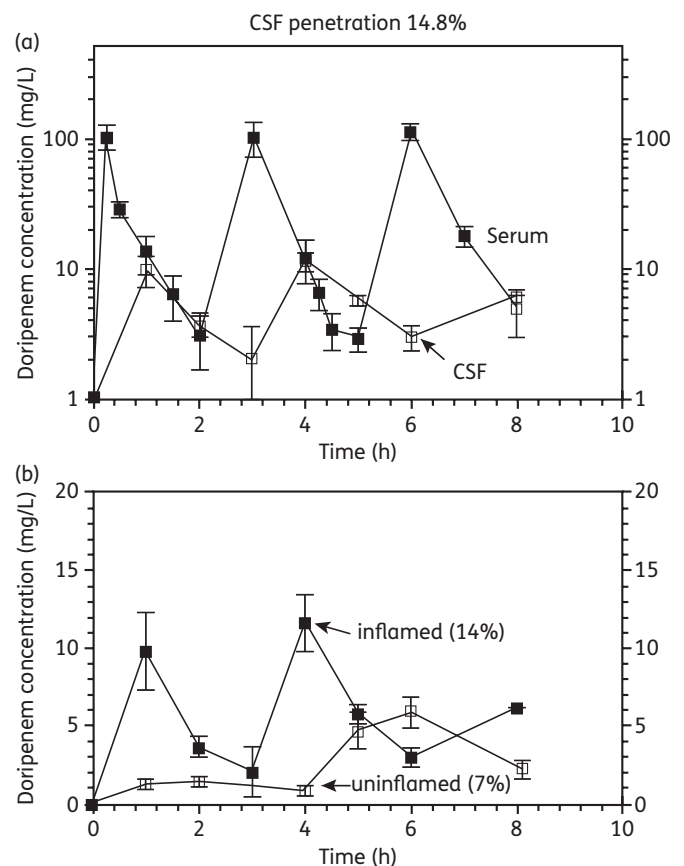


Figure 1. (a) Serum and CSF levels of doripenem after three iv injections of 75 mg/kg. The filled squares represent serum levels and open squares represent CSF levels. Results are expressed as means \pm SD. (b) CSF levels of doripenem through inflamed meninges (filled squares) and through uninflamed meninges (open squares). Results are expressed as means \pm SD.

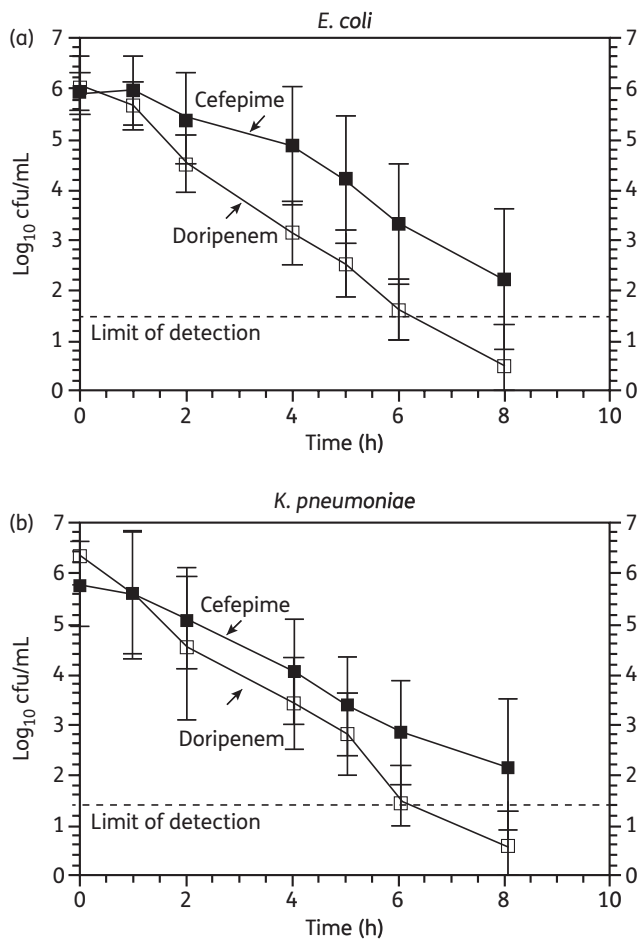


Figure 2. (a) Decrease in the bacterial titre of *E. coli* after three injections of 75 mg/kg doripenem (open squares) and after two injections of 100 mg/kg cefepime (filled squares). Results are expressed as means±SD. The broken line represents the limit of detection of bacteria in the CSF (1.7 log₁₀ cfu/mL). (b) Decrease in the bacterial titre of *K. pneumoniae* after three injections of 75 mg/kg doripenem (empty squares) and after two injections of 100 mg/kg cefepime (filled squares). Results are expressed as means±SD. The broken line represents the limit of detection of bacteria in the CSF (1.7 log₁₀ cfu/mL).

In non-infected rabbits (uninflamed meninges) the CSF levels were about 1 mg/L during the first 4 h and increased progressively to 5 mg/L after the third injection. The kinetics of doripenem through uninflamed and inflamed meninges is shown in Figure 1(b). The penetration of doripenem through inflamed meninges was 14±3.4% and 7±0.68% through uninflamed meninges, determined by comparison of AUCs (GraphPad Prism Software, version 5.0).

The penetration of cefepime into the CSF has been studied previously.¹³ In the present study only CSF concentrations of cefepime were measured, ranging between 10 and 15 mg/L. The first injection led to levels around 10 mg/L, and after the second injection cefepime levels peaked at 16 mg/L, decreasing slowly to 14 mg/L at the end of the experimental period (data not shown).

The MICs of both antibiotics were identical for the two strains, i.e. 0.12 mg/L for *E. coli* and 0.25 mg/L for *K. pneumoniae*.

Figure 2(a and b) reflects the kinetics of the killing activity of the different regimens. Against *K. pneumoniae*, doripenem produced a more rapid decrease in the bacterial titre over 8 h than the comparator. The antibacterial activity of the different regimens is summarized in Table 1. The efficacy is expressed as killing rate per hour and killing rate per 8 h (i.e. at the end of the experimental period). Against *K. pneumoniae*, doripenem produced a higher but marginally significant decrease per hour of the viable cell count than cefepime (−0.73±0.19 log₁₀ cfu/mL versus −0.48±0.09 log₁₀ cfu/mL). At the end of the experimental period the antibacterial activity of doripenem was slightly but not significantly superior to cefepime (−5.40±1.37 log₁₀ cfu/mL versus −3.59±0.89 log₁₀ cfu/mL).

Against *E. coli*, doripenem produced a similar antibacterial activity, with a killing rate per hour around −0.73 log₁₀ cfu/mL and −5.55 log₁₀ cfu/mL over 8 h. In this experimental setting, doripenem was not significantly superior to cefepime over 8 h (killing rate of cefepime over 8 h = −3.80 log₁₀ cfu/mL). Only the killing rate per hour was significantly superior (killing rate of cefepime per hour = −0.47 log₁₀ cfu/mL).

At the end of the experimental period, the CSF samples for 5 of 10 rabbits infected with *K. pneumoniae* in the doripenem group were sterile versus 2 of 10 in the comparator group.

Against *E. coli*, the difference in the decrease in the viable cell count of the two regimens was of the same order of magnitude.

Table 1. Antibacterial activity of the different regimens

Group (n)	Strain	Inoculum at 0 h	ΔKilling/h	ΔKilling/8 h
Control (10)	<i>K. pneumoniae</i>	6.02±0.30 log ₁₀ cfu/mL	+0.12±0.02 log ₁₀ cfu/mL	+1.02±0.22 log ₁₀ cfu/mL
Doripenem (10)	<i>K. pneumoniae</i>	6.37±0.63 log ₁₀ cfu/mL	−0.73±0.19 log ₁₀ cfu/mL ^a	−5.40±1.37 log ₁₀ cfu/mL ^b
Cefepime (10)	<i>K. pneumoniae</i>	5.79±0.83 log ₁₀ cfu/mL	−0.48±0.09 log ₁₀ cfu/mL ^a	−3.59±0.89 log ₁₀ cfu/mL ^b
Control (10)	<i>E. coli</i>	5.95±0.23 log ₁₀ cfu/mL	+0.11±0.05 log ₁₀ cfu/mL	+0.92±0.37 log ₁₀ cfu/mL
Doripenem (10)	<i>E. coli</i>	6.07±0.57 log ₁₀ cfu/mL	−0.73±0.12 log ₁₀ cfu/mL ^c	−5.55±0.87 log ₁₀ cfu/mL ^d
Cefepime (10)	<i>E. coli</i>	5.96±0.38 log ₁₀ cfu/mL	−0.47±0.14 log ₁₀ cfu/mL ^c	−3.80±1.10 log ₁₀ cfu/mL ^d

^aTukey–Kramer multiple comparisons test; doripenem versus cefepime: *P*<0.05.

^bTukey–Kramer multiple comparisons test; doripenem versus cefepime: *P*>0.05, not significant.

^cTukey–Kramer multiple comparisons test; doripenem versus cefepime: *P*<0.05.

^dTukey–Kramer multiple comparisons test; doripenem versus cefepime: *P*>0.05, not significant.

At the end of the experimental period, doripenem managed to sterilize 7 of 10 CSF samples versus 2 of 10 for cefepime.

Discussion

The treatment of nosocomial infections caused by Gram-negative and Gram-positive pathogens remains a major challenge for clinicians and infectiologists, especially since increasing resistance of common clinical pathogens has limited the choice of therapeutic options. In general, carbapenems are the preferred empirical treatment for the usual nosocomial pathogens because of their extended spectrum.

Doripenem is a new member of the carbapenem class of β -lactam antibiotics, with a spectrum of activity against Gram-positive and Gram-negative pathogens, similar to meropenem and imipenem. Against wild-type *Pseudomonas aeruginosa*, doripenem may be slightly more efficacious than the other carbapenems.¹⁶

In our meningitis rabbit model we tested the efficacy of doripenem against two Gram-negative microorganisms (*K. pneumoniae* and *E. coli*) and measured its penetration into the CSF. The comparator regimen was cefepime. The MICs of the two antibiotics were identical for both bacterial strains. In our experimental model, doripenem had to be injected three times (3×75 mg/kg) due to its very short half-life in rabbits. In humans, the half-life of doripenem is about 1 h.⁸ In our study, each injection of doripenem led to a peak serum level of about 100 mg/L, which decreased rapidly to 2 mg/L 2 h later. The CSF levels fluctuated between 10 and 2 mg/L, with a level around 7 mg/L at the end of the experimental period. Despite higher serum peak levels, 75 mg/kg of doripenem injected three times during 8 h mimics approximately the kinetics of one injection of 500 mg in humans.¹⁷ The CSF penetration of doripenem into inflamed meninges was 14%, comparable to meropenem, but higher than ertapenem (7%).^{10,18} The penetration of doripenem into non-inflamed meninges (uninfected animals) was about 7%. During the first 4 h the CSF levels remained low (between 1 and 2 mg/L), but increased for unknown reasons during the second part of the experimental period, with a peak level at about 6 mg/L (see Figure 1b). Cefepime injected twice (2×100 mg/kg) led to CSF levels between 10 and 15 mg/L, similar to levels measured in previous studies.^{12,13} The penetration of cefepime into the CSF of rabbits as determined in previous studies ranged between 16% and 22%.^{19,20} Based on their approximately similar CSF levels and their identical MICs for the two pathogens, comparable CSF level/MIC ratios could be expected for the two antibiotics. The CSF peak level/MIC ratio was 83 for doripenem and 125 for cefepime against *E. coli* and 40 for doripenem and 60 for cefepime against *K. pneumoniae*, respectively. In this study, time over the MIC ($T > \text{MIC}$) was not a good discriminating parameter because it was 100% for both antibiotics.

According to their similar CSF levels, MICs and CSF level/MIC ratios, a comparable antibacterial efficacy could be assumed for doripenem and cefepime. Interestingly, doripenem was more efficacious against both pathogens. Against *K. pneumoniae*, doripenem led to a decrease in the bacterial titre of $5.40 \pm 1.37 \log_{10}$ cfu/mL after 8 h versus $3.59 \pm 0.89 \log_{10}$ cfu/mL for cefepime. The difference was not significant due to the higher

standard deviation in the doripenem group. Doripenem managed to sterilize 5 of 10 CSF samples versus 2 of 10 for cefepime.

Against *E. coli*, the difference between doripenem and cefepime was slightly more pronounced, but not significant. Doripenem managed to sterilize 7 of 10 CSF samples versus 2 of 10 for cefepime.

In summary, this study showed that doripenem, based on its bactericidal activity and good penetration into the CSF, might be a very promising option in the treatment of meningitis caused by nosocomial pathogens, although this disease is not very common. Thanks to its very low seizure potential²¹ and its high efficacy against usual community-acquired pathogens (i.e. pneumococci), doripenem could be an interesting modality in the empirical treatment of community-acquired bacterial meningitis.

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Transparency declarations

J. M. L. is an employee of Janssen EMA. Other authors: none to declare.

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