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In vivo efficacy of ceftolozane against *Pseudomonas aeruginosa* in a rabbit experimental model of pneumonia: Comparison with ceftazidime, piperacillin/tazobactam and imipenem

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ABSTRACT

The aim of this study was to compare ceftolozane with ceftazidime, piperacillin/tazobactam (TZP) and imipenem in an experimental rabbit model of *Pseudomonas aeruginosa* pneumonia. Efficacy was assessed following 2 days of treatment by total colony counts in different tissues (lung, spleen and blood culture). Mean ± standard deviation pulmonary bacterial loads were 4.9 ± 0.3, 3.6 ± 0.3, 4.8 ± 0.2, 5.5 ± 0.8 and 3.9 ± 0.3 $\log_{10}$ CFU/g of lung for ceftolozane (1 g), ceftolozane (2 g), ceftazidime, TZP and imipenem, respectively, compared with 6.3 ± 0.9 $\log_{10}$ CFU/g of lung for control animals. The higher ceftolozane dose [2 g three times daily (t.i.d.)] showed significantly better efficacy than the lower dose (1 g t.i.d.). In conclusion, in this rabbit model of *P. aeruginosa* pneumonia, ceftolozane had an efficacy equivalent to that of comparator agents at a dose of 1 g t.i.d. and had better efficacy at a higher dose (2 g t.i.d.).

1. Introduction

Among patients with healthcare-associated infections, *Pseudomonas aeruginosa* is the most common Gram-negative cause of ventilator-associated pneumonia and is the second most common cause of catheter-associated urinary tract infection (UTI) behind *Escherichia coli* [1]. The increasing frequency of multidrug-resistant strains is of great concern as efficacious antimicrobial options are severely limited [2].

Ceftolozane is a novel antipseudomonal cephalosporin that is being developed in combination with the β-lactamase inhibitor tazobactam to broaden coverage to include β-lactamase-producing Gram-negative organisms. It is being studied for the treatment of complicated intra-abdominal infections (cIAIs), complicated UTIs (cUTIs) and nosocomial pneumonia.

The aim of this study was to compare ceftolozane with ceftazidime, piperacillin/tazobactam (TZP) and imipenem in an experimental rabbit model of *P. aeruginosa* pneumonia. Previous animal as well as human studies are of poor help in determining the dosing regimen providing the greatest efficacy [3–5]. Thus, the aim of this study was also to compare different ceftolozane doses.

2. Materials and methods

2.1. Antimicrobial drugs

Clinical forms of the following antibiotics were used: ceftolozane (Cubist, Lexington, MA); ceftazidime (GlaxoSmith-Kline, Marly-le-Roi, France); TZP (Pfizer, Paris, France); and imipenem/cilastatin (Merck Sharp & Dohme-Chibret, Courbevoie, France). Drugs were dissolved in saline just before administration according to the manufacturers' recommendations.

2.2. 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. Minimum inhibitory concentrations (MICs) were determined by standard agar dilution as recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie [6].

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2.3. Experimental P. aeruginosa pneumonia in rabbits

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

Female New Zealand White immunocompetent rabbits (body weight 2.2–2.5 kg) were obtained from CEGAV (Saint Mars d'Égrenne, France) and were placed in individual cages. Food and water were provided ad libitum. Venous and arterial catheters were inserted and pneumonia was initiated as described previously [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 $\log_{10}$ 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].

Animals were randomly assigned to six groups: (i) no treatment (controls); (ii and iii) ceftolozane groups [computer-controlled syringe pump infusion simulating either a human-equivalent (HE) dose of 1 g three times daily (t.i.d.) or an HE dose of 2 g t.i.d.]; (iv) ceftazidime group (simulating an HE dose of 2 g t.i.d.); (v) TZP group (simulating an HE dose of piperacillin/tazobactam 4 g/0.5 g four times daily); and (vi) imipenem group (simulating an HE dose of 1 g t.i.d.). These simulations reflect typical recommended clinical doses [4]. Seven animals were randomly assigned to each treatment group and 12 animals were assigned to the control group. Antibiotic infusions were stopped 4 h before the animals were euthanised. Expected spontaneous mortality was 20%.

2.4. 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 Difco antibiotic medium 2 (Becton Dickinson, Le Pont-de-Claix, France) and *Bacillus subtilis* ATCC 9466 as the indicator organism (sensitivity threshold of 0.5 mg/L and intraday and interday variations <10%). 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.

2.5. Evaluation of infection

At the end of the treatment (2 days), animals were euthanised following international guidelines. For each lung, quality checks were performed to confirm that the animals actually had invasive infection. Thus, for each animal, a macroscopic score was determined as detailed by Piroth et al. [9]. Histological sections were also systematically performed to confirm the invasive character of the infection. The spleen and both lungs from each animal were weighed and homogenised in 1 mL of saline buffer and were 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 ± standard deviation (S.D.) $\log_{10}$ CFU/g of lung. Blood cultures were also performed. Spleen and blood culture results were expressed qualitatively (positive or negative).

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

2.6. Statistical analysis

Statistical analyses were performed using Microsoft® Excel 2007 (Microsoft Corp., Redmond, WA), PASW Statistics for Windows v.18.0 (SPSS Inc., Chicago, IL) and GraphPad Prism® 4 (GraphPad Software Inc., La Jolla, CA). Results were expressed as the mean ± S.D. Quantitative variables were compared using one-way analysis of variance (ANOVA). This analysis was completed with a post-hoc Bonferroni test. Proportions (percentages) were compared using Fisher's exact test. A *P*-value of <0.05 was considered statistically significant.

3. Results

3.1. Minimum inhibitory concentrations

The MICs for *P. aeruginosa* PAO1 were 0.5 mg/L for ceftolozane, 1 mg/L for ceftazidime, 4 mg/L for TZP or for piperacillin alone and 0.5 mg/L for imipenem.

3.2. Pharmacokinetics

Pharmacokinetic/pharmacodynamic (PK/PD) parameters are summarised in Table 1. The actual exposure curves are presented in Supplementary Figs. S1–S5.

3.3. Comparison of the efficacy of ceftolozane, ceftazidime, piperacillin/tazobactam and imipenem

Of the 12 control animals, 2 died before evaluation. One animal died from each of the ceftazidime and TZP groups and none from the ceftolozane or imipenem groups. These differences were not statistically significant. Data were not available for one animal in the imipenem group because of antibiotic administration error (electric pump failure). The quality controls confirmed the invasiveness of pneumonia and showed no difference between groups.

No resistant mutants were isolated among the surviving bacteria from any treated animal. Mean pulmonary bacterial loads were statistically different versus controls ($P < 0.01$) for all treatment

Table 1
Pharmacokinetic/pharmacodynamic (PK/PD) parameters for the different treatment groups.

PK/PD parameter	Ceftolozane 1 g t.i.d.	Ceftolozane 2 g t.i.d.	Ceftazidime 2 g t.i.d.	Piperacillin 4 g q.i.d.	Imipenem 1 g t.i.d.
C_{max} (mean ± S.D.) (mg/L)	54 ± 6	96 ± 12	84 ± 13	179 ± 24	74 ± 15
$t_{1/2}$ (h)	2.52	2.20	1.57	0.95	1.27
C_{max}/MIC	108	192	84	45	148
$fT_{>MIC}$ (%)	100	100	100	100	100
Estimated AUC (mg h/L)	134	224	163	245	125
AUC/MIC	268	448	163	61	250

t.i.d., Three times daily; q.i.d., four times daily; C_{max}, maximum drug concentration in plasma; S.D., standard deviation; $t_{1/2}$, half-life; MIC, minimum inhibitory concentration; $fT_{>MIC}$, percentage of the dosing interval that the drug concentration remains above the MIC for the infecting pathogen; AUC, area under the concentration–time curve.

Table 2
Pulmonary bacterial load and spleen and blood culture results for the different treatment groups and controls.

	Controls ($n = 10$)	Ceftolozane 1 g t.i.d. ($n = 7$)	Ceftolozane 2 g t.i.d. ($n = 7$)	Ceftazidime 2 g t.i.d. ($n = 6$)	TZP 4 g q.i.d. ($n = 6$)	Imipenem 1 g t.i.d. ($n = 6$)	*P*-value[a]
Mean ± S.D. pulmonary bacterial load ($\log_{10}$ CFU/g)[b]	6.3 ± 0.9	4.9 ± 0.3	3.6 ± 0.3	4.8 ± 0.2	5.5 ± 0.8	3.9 ± 0.3	10^{-6}
Spleen cultures positive/negative[c]	8/2	4/3	2/5	3/3	5/1	2/4	N/S
Blood cultures positive/negative[c]	2/8	0/7	0/7	1/5	1/5	0/6	N/S

t.i.d., Three times daily; TZP, piperacillin/tazobactam; q.i.d., four times daily; S.D., standard deviation; N/S, not significant.

[a] Quantitative variables were compared by one-way analysis of variance (ANOVA); proportions (percentages) were compared using Fisher's exact test.

[b] Bacterial loads were significantly different for all antibiotics compared with the controls, except TZP. Post-hoc Bonferroni test revealed no difference between the 1 g ceftolozane group and the ceftazidime, TZP or imipenem groups, whereas the 2 g ceftolozane group had a significantly lower bacterial load than both ceftazidime ($P < 0.05$) and TZP ($P < 0.01$) groups.

[c] No difference was observed for spleen or blood cultures between the groups. The minimum inhibitory concentrations (MICs) for *P. aeruginosa* PAO1 were 0.5 mg/L for ceftolozane, 1 mg/L for ceftazidime, 4 mg/L for TZP and 0.5 mg/L for imipenem.

groups, except TZP; surprisingly, for the TZP group the reduction in bacterial pulmonary load did not reach significance.

However, the pulmonary bacterial load did not differ significantly when ceftolozane (1 g t.i.d.) and reference regimens were compared (Table 2). Results for bacterial pulmonary load are also shown in Supplementary Fig. S6.

Table 2 also shows the results of spleen cultures for the different groups. Spleen cultures were more often positive in the TZP group but this difference did not reach statistical significance. There was also no difference in blood culture.

3.4. Efficacy of the higher dose of ceftolozane

Testing a higher dose of ceftolozane (simulating an HE dose of 2 g t.i.d.) showed significantly higher efficacy in reducing bacterial pulmonary load than the lower dose of ceftolozane but also than ceftazidime or TZP. In the present study, imipenem has an interesting efficacy. For the group treated with this agent, we did not reveal any difference with the ceftolozane groups.

4. Discussion

This study provides efficacy data for ceftolozane compared with reference drugs in a rabbit experimental model of *P. aeruginosa* pneumonia.

Ceftolozane/tazobactam is a novel antibacterial with activity against *P. aeruginosa*, including drug-resistant strains, as well as other common Gram-negative pathogens including most extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. Currently, ceftolozane/tazobactam is being studied in phase three trials for the treatment of cUTIs and cIAIs at the 1.5 g dose and nosocomial pneumonia at the 3 g dose. The activity of ceftolozane against *P. aeruginosa* is not affected by the addition of tazobactam because ESBLs are uncommon in *P. aeruginosa* and ceftolozane is stable to the AmpC β-lactamases.

The in vitro activity of ceftolozane is of particular interest against carbapenem-resistant and multidrug-resistant *P. aeruginosa* or ESBL-producing bacteria [10,11]. The strong affinity of ceftolozane for the *P. aeruginosa* penicillin-binding proteins might also be of interest compared with imipenem [12]. In addition, ceftolozane is not a substrate of the MexAB–OprM, MexCD–OprJ, MexEF–OprN and MexXY efflux pumps or the carbapenem-specific porin OprD [13]. Furthermore, antibiofilm and resistance suppression activities against PAO1 chronic respiratory infections have been described [14].

The present data confirm the efficacy of ceftolozane in a rabbit experimental pneumonia model and demonstrate comparable efficacy at even the lower ceftolozane dose against comparators tested at highest recommended doses such as simulated 2 g t.i.d. for ceftazidime or 4 g/0.5 g four times a day for piperacillin/tazobactam. The model used was consistent in terms of reproducibility (all animals had positive lung cultures; ca. 60% had positive spleen cultures) and also severity (20% mortality among non-treated animals, bloodstream infections, positive spleen cultures). This experimental model of *P. aeruginosa* pneumonia is also of interest because it allows simulation of human pharmacokinetics: maximum drug concentration in plasma (C_{max}), half-life ($t_{1/2}$) and area under the concentration–time curve (AUC) were all consistent with available human data [4].

For β-lactam antibiotics, the fraction of time during the dosing interval that the drug concentration remains above the MIC for the infecting pathogen ($fT_{>MIC}$) is considered the target that best relates (directly) with patient outcomes [15]. PK/PD parameters that best predict the efficacy of ceftolozane remain uncertain. Indeed, it is surprising to achieve greater efficacy in the 2 g t.i.d. ceftolozane group compared with the 1 g t.i.d. ceftolozane group while in both groups $fT_{>MIC}$ is 100%. Would it be necessary to obtain concentrations as high as 4× or 6× MIC as has been suggested by some authors [16,17]? The current study was unfortunately not designed to answer this question precisely.

These results confirm the efficacy of ceftolozane in comparison with ceftazidime, TZP and imipenem. This could support that the high-dose regimen would be better to treat more severe or difficult-to-treat infections such as those in intensive care units or those developing among cystic fibrosis patients [18].

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Competing interests: None declared.

Ethical approval: The experimental protocol was approved by the Committee of Animal Ethics of the University of Nantes (Nantes, France). Animals were treated in accordance with national regulations on animal studies.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijantimicag.2014.04.017.

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