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Efficacy of ceftolozane in a murine model of *Pseudomonas aeruginosa* acute pneumonia: *in vivo* antimicrobial activity and impact on host inflammatory response

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Objectives: To assess the activity of ceftolozane, a novel oxyimino-cephalosporin, in comparison with ceftazidime and piperacillin/tazobactam against a multidrug-resistant *Pseudomonas aeruginosa* strain using a murine model of pneumonia.

Methods: Quantitative bacteriology, survival, histological examination, myeloperoxidase activity, proinflammatory cytokine levels in lungs and endothelial permeability were evaluated to determine the effects of ceftolozane and comparators on *P. aeruginosa*-induced pneumonia.

Results: After 48 h of treatment, ceftolozane reduced the bacterial load by 3–4 $\log_{10}$ cfu/g of lung. Systemic dissemination of the pulmonary infection and development of lung damage were inhibited in all β-lactam-treated animals. *P. aeruginosa*-induced pneumonia led to elevated concentrations of tumour necrosis factor-α, interleukin (IL)-1β and macrophage inflammatory protein (MIP)-2 in the lungs. While the levels of proinflammatory cytokines decreased following ceftazidime and piperacillin/tazobactam therapy, ceftolozane exhibited increased concentrations of IL-1β and MIP-2 after 24 h of infection, resulted in significantly increased levels of recruited neutrophils within the infected lung without increasing lung endothelial permeability.

Conclusions: These data strongly support ceftolozane as an effective option for the treatment of severe *P. aeruginosa* respiratory infections by improving the early pulmonary inflammatory response without impairing 48 h post-infection homeostasis.

Keywords: cephalosporins, lung, inflammation, cytokines

Introduction

Pseudomonas aeruginosa represents the most common pathogen responsible for both acute respiratory infections in ventilated or immunocompromised patients and chronic respiratory infections in cystic fibrosis patients. In addition to high intrinsic resistance to many antimicrobial agents, *P. aeruginosa* exhibits an exceptional ability to accumulate additional mechanisms of antibiotic resistance, including membrane permeability, efflux pump systems and mutations in antimicrobial targets.[1] Ceftazidime, introduced 25 years ago, is usually considered as the first-line therapy in the management of *P. aeruginosa* infections, but clinical failures have been reported due to the emergence of constitutive AmpC β-lactamase-producing resistant mutants during treatment.[2] Reports of *P. aeruginosa* strains resistant to almost all available antibiotics highlight the crucial need for new antibiotic therapies for *P. aeruginosa* infections.[3] Nevertheless, a rapid review of the antimicrobial agents in development targeting multidrug-resistant (MDR) *P. aeruginosa* shows that the pipeline of new drug candidates is very limited.

Ceftolozane (previously CXA-101 and FR264205) is a novel antipseudomonal cephalosporin (currently in Phase 3 clinical development), which is being developed for the treatment of certain serious Gram-negative bacterial infections. Coadministration with the well-known β-lactamase inhibitor tazobactam enhances the activity by protecting ceftolozane from hydrolysis and expands the coverage to Enterobacteriaceae, including extended-spectrum β-lactamase-producing strains.[4] Nevertheless, ceftolozane might be viewed as a potent antipseudomonal agent with an antibacterial spectrum against other organisms similar to that of ceftazidime.[4,5] The oxyimino-cephalosporin seems to be less susceptible to the most prevalent mechanisms of ceftazidime resistance, including overexpression of efflux and

derepressed AmpC.[6,7] In this context, this drug could be useful in the treatment of *P. aeruginosa* infections, including nosocomial pneumonia.

The first steps in pneumonia result from the defeat of the innate immune defence system by the infection, and innate immunity is essential in host defence against virulent pathogens, including *P. aeruginosa*.[8] Consequently, it makes sense to assess the *in vivo* activity of antimicrobial agents in pneumonia without overlooking the role of the lung inflammatory status and the impact of the studied drugs on the host immune response.

The aims of the present study were: (i) to assess and compare the *in vivo* activity of the new cephalosporin, ceftolozane, versus ceftazidime and piperacillin/tazobactam using a murine model of *P. aeruginosa* experimental pneumonia; (ii) to investigate the inflammatory status of the lung during experimental *P. aeruginosa* pneumonia; and (iii) to evaluate the impact of the studied drugs on the lung inflammatory status and on surrogate markers of lung damage.

Materials and methods

Bacterial strain

The *P. aeruginosa* strain was a clinical MDR strain isolated from bronchoalveolar lavage exhibiting both a MexAB-OprM efflux system and alteration of porin OprD (imipenem MIC=16 mg/L). The strain was grown overnight in brain heart infusion broth at 37°C (Becton-Dickinson, Franklin Lakes, NJ, USA). Immediately before use, the cell pellet (centrifuged at 1000 **g** for 10 min) was washed twice using 0.9% NaCl. After the second wash, the pellet was resuspended in sterile saline and the inoculum was calibrated by nephelometry.

Antibiotics

Ceftolozane was provided by Cubist, Inc. Clinical forms of ceftazidime and piperacillin/tazobactam (generic formulation) were used in this study and were supplied by GlaxoSmithKline® (Marly-le-Roi, France) and Teva® (Paris, France), respectively.

Susceptibility testing

The MICs of ceftolozane, ceftazidime, piperacillin/tazobactam, imipenem and meropenem were determined in cation-supplemented Mueller–Hinton broth by the CLSI microdilution technique.[9]

Animals

Six-week-old pathogen-free RjOrl:SWISS mice (weight, 20–24 g) were purchased from Janvier Laboratories (Le Genest Saint Isle, France). The mice were given food and water *ad libitum*. The animals were treated in accordance with institutional policies and the guidelines stipulated by the animal welfare committee. The Committee of Animal Ethics of the University of Nantes approved all animal experimentation in this study.

Pharmacokinetic studies

The doses used for each of the study drugs were designed to achieve plasma levels similar to the AUC values obtained with intravenous formulations of ceftolozane (1 g thrice daily), ceftazidime (1 g thrice daily) and piperacillin/tazobactam (4 g thrice daily) in humans.[10–12] The amount of protein binding in mouse serum is similar to that of protein binding in human serum for ceftolozane, ceftazidime and piperacillin/tazobactam.[13–15] To assess the pharmacokinetics of increasing doses of drugs in mice, blood was collected into a heparinized syringe (heparin, 5 U; Sanofi Winthrop, Gentilly, France) by intracardiac puncture in mice [anaesthetized with isoflurane (Abbott, Chicago, IL, USA)] at different times after subcutaneous (sc) injections. After pharmacokinetic assessment of increasing doses of the studied drugs, the doses required to simulate the AUC values obtained in humans were 180 mg/kg thrice daily, 200 mg/kg thrice daily and 400 mg/kg thrice daily for ceftolozane, ceftazidime and piperacillin/tazobactam, respectively. Concentrations of ceftolozane in plasma were determined by microbiological assay with *Klebsiella pneumoniae* as the test organism and Antibiotic Medium no. 2 (Difco Laboratories, Detroit, MI, USA) as the diffusion medium (lower detection limit, 0.5 mg/L; intra- and interday variations, <10%). Concentrations of both ceftazidime and piperacillin/tazobactam were determined by microbiological assay with *Bacillus subtilis* ATCC 9466 as the test organism and Antibiotic Medium no. 2 as the diffusion medium (lower detection limit, 0.5 mg/L; intra- and interday variations, <10%).

Pneumonia model

Pneumonia was induced as previously described.[16] Mice were briefly anaesthetized with isoflurane (Abbott, Chicago, IL, USA) and placed in dorsal recumbency. Transtracheal insertion of a 24 gauge feeding needle was used to inject 70 μL of a bacterial suspension adjusted to 10^9 cfu/mL. Treatment was started 2 h after the bacterial challenge and the antibiotics were administered to the animals using the sc route for 2 days.

Therapeutic regimens

Animals were assigned to four groups: no treatment (controls) and sc injection (every 8 h) of ceftolozane, ceftazidime and piperacillin/tazobactam.

Survival of *P. aeruginosa*-infected mice

To determine the effects of ceftolozane, ceftazidime and piperacillin/tazobactam on the survival of *P. aeruginosa*-infected mice, mice were infected with *P. aeruginosa*, treated with either saline or the studied drugs starting 2 h post-infection and monitored at 3, 6, 12, 24 and 48 h after the bacterial challenge (treatment period).

Bacteriological assessment of infected lung and bacterial dissemination (spleen cultures)

Lungs and spleen from each animal were removed, weighed and homogenized in 1 mL of saline buffer (Mixer Mill MM 400; Retsch Inc., Newtown, PA, USA) and used for quantitative cultures on agar for 24 h at 37°C. Serial dilutions were performed and cultured at 37°C. Viable counts, after 24 h of incubation, were expressed as the mean ± SD $\log_{10}$ cfu per gram of organ. To determine whether ceftolozane, ceftazidime and piperacillin/tazobactam regimens could induce the selection of *in vivo* resistant variants, undiluted lung homogenates were spread on agar plates containing ceftolozane, ceftazidime and piperacillin/tazobactam at concentrations corresponding to 4-fold the MIC. Bacterial counts were determined after 48 h of incubation at 37°C.

Histological analysis

At 0, 24 and 48 h post-infection, groups of three mice were euthanized and both lungs were removed and immediately placed in 4% formalin.

Formalin-fixed tissues were processed, stained with haematoxylin and eosin, and then analysed by microscopy.

Myeloperoxidase (MPO) activity

The MPO assay was performed as previously described.[16] At 24 and 48 h after the bacterial challenge, animals were euthanized and the lungs were removed, frozen in liquid nitrogen and stored at −80°C until assay. Then, the lungs were mechanically homogenized (Mixer Mill MM 400) in 1 mL of potassium phosphate (50 mM) with *N*-ethylmaleimide (10 mM). The homogenate was washed twice (centrifuged at 12 000 **g** for 30 min at 4°C), suspended in 1 mL of potassium phosphate buffer (50 mM) containing 0.5% of hexadecyl trimethylamonium and sonicated on ice water for 3 min. Heat shock was performed for 2 h at 60°C and then samples were centrifuged at 12 000 **g** for 10 min. The H_2O_2-dependent oxidation of *o*-dianisidine was determined by measuring absorbance at 460 nm. Supernatant MPO activity was normalized to lung weight.

Preparation of lung homogenate for ELISA and determination of cytokine levels

Immediately after removal, weighed lung samples were mechanically homogenized in cold lysis buffer (1× PBS, pH 7.4/0.1% Triton X-100) containing a 1 mM protease inhibitor cocktail (Sigma, St Louis, MO, USA). Tumour necrosis factor (TNF)-α, interleukin (IL)-1β and macrophage inflammatory protein (MIP)-2 concentrations in lung homogenate were quantified with ELISA kits according to the manufacturer's instructions (R&D Systems, Lille, France). The protein concentration in each sample was determined using the BCA™ protein assay kit (Pierce, Rockford, IL, USA).

Lung endothelial permeability

The determination of the lung endothelial permeability was performed as previously described by Boutoille *et al.*[17] Briefly, mice were given a 2 mg intraperitoneal injection of fluorescein isothiocyanate (FITC)-conjugated albumin (Sigma, Lyon, France). Two hours later, the lungs were removed, mechanically homogenized in 1 mL of 0.9% NaCl and then centrifuged at 4000 **g** for 10 min. Blood was collected via a right ventricular puncture and centrifuged at 4000 **g** for 10 min. FITC-albumin was measured in 100 μL aliquots of supernatant obtained from lung homogenates and blood by fluorimetry at 480 nm.

Statistics

Statistical analyses were performed with GraphPad Prism software (version 4.0; GraphPad Software, San Diego, CA, USA). Normally distributed data were analysed using analysis of variance to compare the effects between the different groups, followed by a Bonferroni's test to compare the treated groups two by two. Continuous non-parametric variables were expressed as median (IQR) and were compared using the Kruskal–Wallis test for multiple comparisons. In case of significance, the Mann–Whitney test was used for intergroup comparison. $P<0.05$ was considered significant.

Results

Ceftolozane shows superior antibacterial activity in P. aeruginosa-induced pneumonia

The MIC values of ceftolozane, ceftazidime, piperacillin/tazobactam, imipenem and meropenem were 1, 4, 64, 16 and >32 mg/L, respectively. Ceftolozane had the lowest MIC for this MDR *P. aeruginosa* strain. The pharmacokinetic/pharmacodynamic parameters obtained after sc injections of the studied drugs are summarized in Table 1. After 24 h (data not shown) and 48 h of infection, ∼7 $\log_{10}$ cfu/g was recovered from the infected lung. The bacterial load in the spleen was ∼5 $\log_{10}$ cfu/g, suggesting an important systemic dissemination of infection from the infected lung tissues (Table 2). Ceftolozane demonstrated excellent *in vivo* bactericidal activity after 48 h of treatment and was able to decrease the lung bacterial load by 3–4 $\log_{10}$ cfu/g of tissue. As expected, ceftolozane was more effective than the combination piperacillin/tazobactam against *P. aeruginosa* ($P<0.05$), but, surprisingly, piperacillin/tazobactam achieved significant activity in infected lung despite the high MIC for the strain (i.e. 64 mg/L). By 48 h, ceftazidime-treated mice showed a significant decrease in the lung bacterial burden as compared with the controls, but ceftolozane remained more active *in vivo* ($P<0.05$). The spleen burden after 48 h of treatment with ceftolozane, ceftazidime or piperacillin/tazobactam was inferior to 3 $\log_{10}$ cfu/g. The decrease in the bacterial load in the spleen was similar between the studied β-lactams and seemed to be unrelated to their respective MICs. None of the regimens studied after 48 h of incubation at 37°C showed *P. aeruginosa* colonies on agar plates containing the studied drugs at four times the MIC.

As shown in Figure 1, *P. aeruginosa*-induced pneumonia displayed an overall mortality of 70%, with a steady decrease in

Table 1. Pharmacokinetic/pharmacodynamic parameters for the different treatment groups (ceftolozane, ceftazidime and piperacillin/tazobactam)

	Ceftolozane	Ceftazidime	Piperacillin/tazobactam
Dose (mg/kg)	180	200	400
C_{max} (mg/L), mean ± SD	177.4 ± 18.4	170.3 ± 22.5	189.0 ± 21.6
T_{max} (h:min)	0.17:10	0.17:10	0.33:20
Estimated AUC (mg·h/L)	144.5	134.7	114.7
Percentage of time above MIC	34	22	10

Table 2. Bacterial counts in lung and spleen after 48 h of treatment with ceftolozane, ceftazidime and piperacillin/tazobactam

	$\log_{10}$ cfu/g of organ, mean ± SD	
Regimen	lung	spleen
Controls	7.05 ± 0.86	5.06 ± 0.63
Ceftolozane	3.61 ± 0.35[a,b]	2.63 ± 0.46[a]
Ceftazidime	4.74 ± 1.01[a]	2.74 ± 0.49[a]
Piperacillin/tazobactam	5.04 ± 0.90[a]	2.80 ± 0.84[a]

[a]$P<0.001$ versus controls.
[b]$P<0.05$ versus ceftazidime and piperacillin/tazobactam groups.

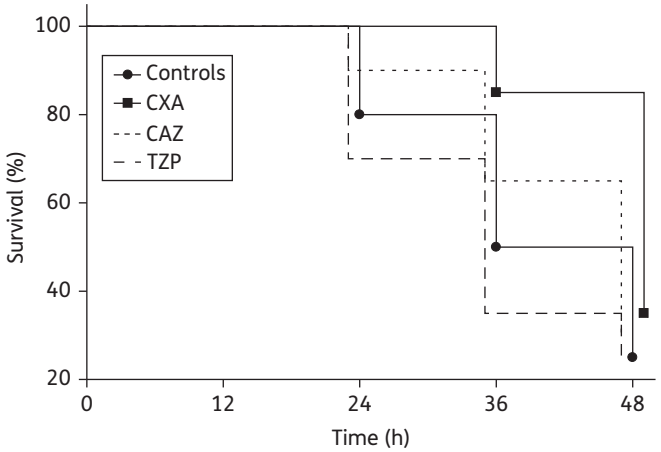

Figure 1. Mice survival over 48 h following intratracheal inoculation of *P. aeruginosa* and treatment with ceftolozane (CXA), ceftazidime (CAZ) or piperacillin/tazobactam (TZP). Survival rates are expressed as percentages and are representative of three independent experiments (each group, $n=8$).

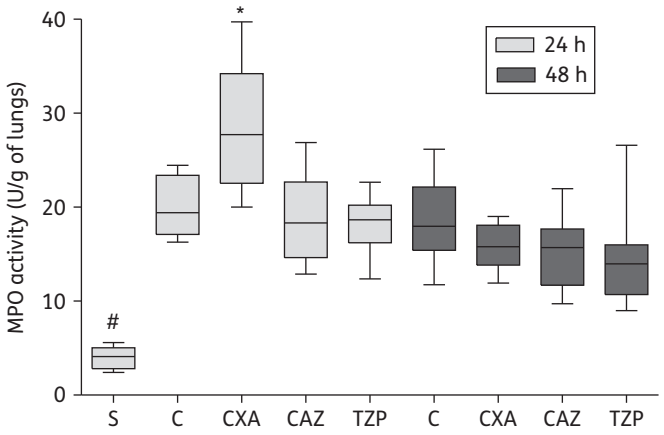

Figure 2. Neutrophil accumulation in infected lung tissues (assessed by MPO activity) after 24 and 48 h of infection. Five groups of mice were studied: sham-treated (S; non-infected, no treatment); untreated (C; control group); ceftolozane-treated mice (CXA group); ceftazidime-treated mice (CAZ group); and piperacillin/tazobactam-treated mice (TZP group). Boxes represent the median (IQR). Data are representative of three independent experiments (each group, $n=6$). #$P<0.001$ versus all other groups. *$P<0.01$ versus all other groups.

survival from 24 to 48 h post-infection confirming this model as an acute model of pneumonia. Despite antibacterial treatment, survival was not significantly different between the control mice and the treated mice ($P>0.05$). The hazard ratios for survival in the treated animals versus the control group were 1.64 (95% CI, 0.87–5.82; $P=0.0948$), 1.25 (95% CI, 0.57–3.48; $P=0.4584$) and 0.87 (95% CI, 0.33–1.96; $P=0.6342$) for ceftolozane, ceftazidime and piperacillin/tazobactam, respectively.

Immune cell infiltrates are detected after 24 and 48 h in infected lung tissues

Lung histopathological examination at 24 h showed that all mice displayed mild pulmonary inflammation that slightly increased at 48 h with an enhanced percentage of lungs affected (Figure S1, available as Supplementary data at *JAC* Online). In the sham group, lung tissue was characterized by thin-walled air spaces with a single pneumocyte layer (Figure S1A). Immune cell infiltrates were detected at both 24 and 48 h post-infection, with macrophages and neutrophils within the alveoli and bronchiolar lumen. At 48 h post-infection, the alveolar spaces were progressively filled up with an increasing number of inflammatory cells (Figure S1B and C). No difference was observed between the treatment groups (data not shown).

Impact of β-lactam therapy on neutrophil-mediated inflammation

To assess neutrophil accumulation in the infected lung tissues, we determined MPO activity after 24 and 48 h of infection. The MPO levels detected in *P. aeruginosa*-infected mice confirmed the inflammation status of the lungs during bacterial pneumonia up to 48 h post-infection (Figure 2). Antibacterial therapy exhibited a very low impact on MPO levels, with generally no significant differences between the untreated and treated groups after both 24 and 48 h of infection. Of interest, increased levels of MPO were observed at 24 h post-infection in ceftolozane-treated animals.

Ceftolozane, ceftazidime and piperacillin/tazobactam decrease endothelial lesions in infected mice

Neutrophil recruitment into the lungs is critical for bacterial clearance, but may also alter endothelial cells and cause acute lung injury/acute respiratory distress syndrome.[18] To investigate the impact of antibiotics on the development of endothelial lesions during the pneumonia process, the lung endothelial permeability was determined after 24 and 48 h of infection. *P. aeruginosa*-infected mice showed a time-dependent increase in endothelial permeability to FITC-albumin, confirming the development of lung lesions during acute *P. aeruginosa* pneumonia (Figure 3). A dramatic decrease in endothelial permeability was detected in animals treated with ceftolozane, ceftazidime and piperacillin/tazobactam as compared with the control group 48 h post-infection, suggesting a protective effect of the drugs on the development of pulmonary oedema.

Ceftolozane increases both proinflammatory cytokine IL-1β and chemokine MIP-2 levels at 24 h post-infection, but not at 48 h

High levels of cytokines were observed after 24 h of infection in infected mice and decreased at 48 h, as expected, with no difference between treated and untreated groups (Figure 4). At 24 h, ceftazidime and piperacillin/tazobactam decreased TNF-α, IL-1β and MIP-2 concentrations in lung homogenates as compared with the concentrations observed in control animals. Of interest, ceftolozane demonstrated the opposite effect by increasing IL-1β and MIP-2 levels in lung 24 h after the bacterial

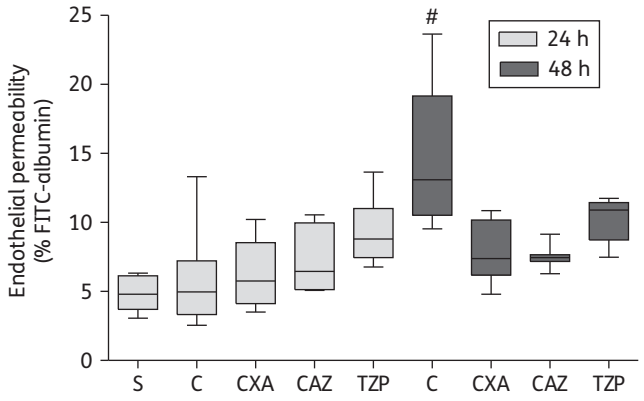

Figure 3. Vascular permeability assessed by measuring FITC-albumin in lung homogenates of infected mice. Five groups of mice were studied: sham-treated (S; non-infected, no treatment); untreated (C; control group); ceftolozane-treated mice (CXA group); ceftazidime-treated mice (CAZ group); and piperacillin/tazobactam-treated mice (TZP group). Data are representative of three independent experiments (each group, $n=6$). Boxes represent the median (IQR). $^{\#}P<0.01$ versus S, C (24 h), CXA (24 h), CAZ (24 h), CXA (48 h) and CAZ (48 h).

challenge. However, basal levels were observed at 48 h, similar to those obtained in the other groups.

Discussion

Therapeutic options for MDR *P. aeruginosa* infections, such as pneumonia, are extremely limited and clinical strains resistant to all available antibiotics have been described.[19] Pharmaceutical companies have mainly focused on Gram-positive bacteria over the last 15 years and the situation is definitely more worrying with Gram-negative pathogens. In this context, it was of special interest to assess the *in vivo* activity of a new potent therapeutic option, ceftolozane (the antibacterial component of the combination drug in clinical development, ceftolozane/tazobactam), using an experimental model close to the pathogenesis of *P. aeruginosa* infections.

Ceftolozane demonstrated excellent *in vivo* efficacy against *P. aeruginosa* as inferred from the significantly lower lung bacterial counts in treated mice when compared with control animals and ceftazidime and piperacillin/tazobactam regimens. Ceftolozane appears more stable against the most common resistance mechanisms driven by mutation in *P. aeruginosa*, such as the hyperproduction of chromosomal cephalosporinase AmpC,[6,7] up-regulation of efflux pumps[20] and repression or inactivation of the porin OprD (involving resistance to carbapenems).[20] Using both *in vitro* mutants and clinical strains, it was demonstrated that CXA-101 retained activity against carbapenem-resistant *P. aeruginosa* (95.3% of 236 isolates showing an MIC of ≤8 mg/L) as well as MDR strains (42% of all tested isolates).[21] In addition, *P. aeruginosa* mutants isolated after antipseudomonal treatment of intensive care unit patients exhibited CXA-101 MICs ≤4 mg/L, despite the emergence of resistance to cephalosporins and carbapenems.[22] By significantly reducing the lung burden of *P. aeruginosa* and demonstrating superior activity in this respiratory infection, ceftolozane demonstrated its potency in the management of *P. aeruginosa* infections. Moreover,

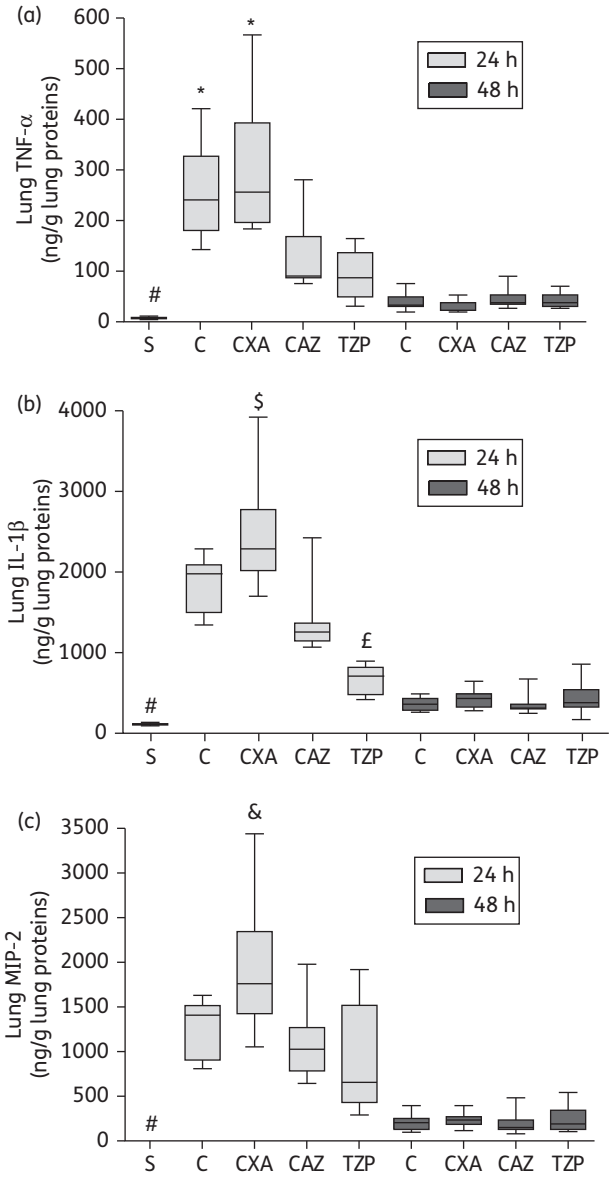

Figure 4. Lung homogenate levels of proinflammatory cytokines, TNF-α and IL-1β, and chemokine, MIP-2, after 24 and 48 h of *P. aeruginosa*-induced pneumonia. Five groups of mice were studied: sham-treated (S; non-infected, no treatment); untreated (C; control group); ceftolozane-treated mice (CXA group); ceftazidime-treated mice (CAZ group); and piperacillin/tazobactam-treated mice (TZP group). Data are representative of three independent experiments (each group, $n=6$). Boxes represent the median (IQR). $\#P<0.05$ versus C (24 h), CXA (24 h) and CAZ (24 h) groups. $^{*}P<0.001$ versus S, CAZ (24 h), TZP (24 h) and all 48 h groups. $\$P<0.01$ versus C (24 h), CAZ (24 h), TZP (24 h) and all 48 h groups. $£P<0.001$ versus C (24 h), CXA (24 h) and CAZ (24 h) groups. $\&P<0.05$ versus all other groups.

ceftolozane significantly improved the survival of infected mice after 24 and 36 h of infection, whereas ceftazidime and piperacillin/tazobactam displayed no effect. Spleen bacterial counts are considered an appropriate marker of the systemic dissemination of the infection. No difference was observed between

therapeutic groups, despite high discrepancies in terms of the MICs. These data suggest a poor correlation between MICs and the *in vivo* activity of β-lactams in both infected lung and spleen. Moreover, the *in vivo* activity of piperacillin/tazobactam against a resistant isolate suggests that a bacteriostatic effect resulting from subinhibitory concentrations of a drug could be sufficient to induce an *in vivo* response leading to an effective therapeutic action.

MPO is a marker enzyme for polymorphonuclear granulocytes (critical contributors to host defence in the lungs),[23] and measurement of MPO activity is an easy and appropriate method to assess recruited neutrophil accumulation in lung tissue.[24] Neutrophil influx in infected lung was detected 3 h after the bacterial challenge (data not shown), confirming that the initial phase of bacterial pneumonia is characterized by neutrophil-mediated inflammation.[24] Antimicrobial agents showed a limited impact on the inflammation status of the lung, with generally no difference observed as compared with control animals. Of interest, ceftolozane-treated animals seem to have the ability to increase the number of recruited neutrophils at the infection site, as shown by increased MPO levels (after 24 h of infection). *P. aeruginosa* is able to impair neutrophil bactericidal functions and to accelerate neutrophil death, as shown by Bianchi *et al*.[25] in a murine model of *P. aeruginosa* pneumonia. Consistent with these data, the higher significant killing of *P. aeruginosa* by the ceftolozane regimen could explain, at least in part, the increased MPO levels. Nevertheless, other mechanisms should be involved in the transient increase in MPO observed in ceftolozane-treated animals.

Of interest, at 48 h, no impact of antibiotic therapy was observed on MPO levels as compared with the untreated control group. Phagocytosis of inflammatory neutrophils by macrophages enables the control of MPO and fosters lung healing by promoting the resolution of tissue injury.[26] The fast clearance of neutrophils in the ceftolozane group may thus appear an adequate response to infection.

In order to further understand the inflammation process during *P. aeruginosa*-induced pneumonia, major proinflammatory cytokines (TNF-α and IL-1β) involved in the acute phase of inflammation as well as the MIP-2 chemokine (considered as a functional homologue of human IL-8 in mice) were investigated.[27] Based on ELISA of lung homogenates, we found, as expected, increased levels of TNF-α, IL-1β and MIP-2 after 24 h of infection,[28,29] followed by a dramatic decrease at 48 h. As shown in Figure 4, β-lactams are able to modulate the *in vivo* production of proinflammatory cytokines and chemokines. The administration of ceftazidime or piperacillin/tazobactam is associated with decreased concentrations of TNF-α, IL-1β and MIP-2 at 24 h (as compared with infected control animals), while ceftolozane-treated animals show elevated levels of both IL-1β and MIP-2 (24 h post-infection). These data are consistent and can explain, in part, the enhanced influx of neutrophils in animals treated with ceftolozane by increasing both the expression of cell adhesion molecules and neutrophil migration to sites of inflammation/infection.[30] Carbapenems have been found to interact with the immune response in a *K. pneumoniae* pneumonia model close to the experimental model used in the present study.[29] Hilliard *et al*.[29] demonstrate that doripenem treatment, but not meropenem or imipenem treatment, resulted in significantly increased IL-6 levels in lung homogenates relative to those in lung homogenates of untreated controls, which may contribute to enhance the neutrophil killing of bacteria in the lung. Moreover, doripenem was associated with improved *in vivo* efficacy relative to meropenem and imipenem treatment. These results are similar to our observations where ceftolozane combines both enhanced *in vivo* efficacy and increased levels of IL-1β and MIP-2, resulting in a greater recruitment of neutrophils in infected lung without increasing the endothelial permeability (a surrogate marker of lung oedema).

The increase in endothelial permeability is a broad indicator of lung injury in murine models.[31] Our findings confirmed that *P. aeruginosa*-induced pneumonia led to a time-dependent increase in endothelial permeability. Two antimicrobial systems have been described in neutrophils and were categorized as oxidative and non-oxidative.[32] The oxidative system catalyses the generation of toxic oxidants. In inflamed situations, MPO may inadvertently damage host tissues.[33] Our results indicate that the increased recruitment of neutrophils in infected lung tissues is not correlated with the development of lung damage in this experimental model of pneumonia. Finally, it seems that therapy with ceftolozane can prevent, at least in part, the development of lung damage during the pneumonia process by an early increase in the lung inflammatory response against *P. aeruginosa* without inducing oedema and by restoring a lung inflammatory homeostasis 48 h post-infection. Although further studies are needed to better understand the differences observed between the studied drugs, the intrinsic properties of antibiotics cannot be excluded, such as specific inhibition of penicillin-binding proteins and morphological alterations as recently highlighted by Moyá *et al*.[34]

In conclusion, the *P. aeruginosa*-induced pneumonia model provides evidence that ceftolozane significantly reduces bacterial counts in lung, decreases the bacterial dissemination of the infection, prevents the development of lung damage and allows the recruitment of more neutrophils at the site of infection by probably increased production of IL-1β and MIP-2. Given that the early recruitment of neutrophils into the lung is critical for initiating an efficient host defence against respiratory pathogen infection,[35] this response of the immune system is likely to be a positive interaction. According to the data presented here, ceftolozane/tazobactam appears to be a promising and effective option for the treatment of severe *P. aeruginosa* infections, including infections caused by multiresistant strains.

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Transparency declarations

None to declare.

Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Livermore DM. Of *Pseudomonas*, porins, pumps and carbapenems. *J Antimicrob Chemother* 2001; **47**: 247–50.

2 Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? *Clin Infect Dis* 2002; **34**: 634–40.

3 Maltezou HC. Metallo-β-lactamases in Gram-negative bacteria: introducing the era of pan-resistance? *Int J Antimicrob Agents* 2009; **33**: 405.e1–7.

4 Livermore DM, Mushtaq S, Ge Y. Chequerboard titration of cephalosporin CXA-101 (FR264205) and tazobactam versus β-lactamase-producing Enterobacteriaceae. *J Antimicrob Chemother* 2010; **65**: 1972–4.

5 Giske CG, Ge J, Nordmann P. Activity of cephalosporin CXA-101 (FR264205) and comparators against extended-spectrum-β-lactamase-producing *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 2009; **64**: 430–1.

6 Takeda S, Ishii Y, Hatano K *et al*. Stability of FR264205 against AmpC β-lactamase of *Pseudomonas aeruginosa*. *Int J Antimicrob Agents* 2007; **30**: 443–5.

7 Takeda S, Nakai T, Wakai Y *et al*. *In vitro* and *in vivo* activities of a new cephalosporin, FR264205, against *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2007; **51**: 826–30.

8 Sadikot RT, Blackwell TS, Christman JW *et al*. Pathogen–host interactions in *Pseudomonas aeruginosa* pneumonia. *Am J Respir Crit Care Med* 2005; **171**: 1209–23.

9 Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Ninth Edition: Approved Standard M07-A9*. CLSI, Wayne, PA, USA, 2011.

10 Ge Y, Whitehouse MJ, Friedland I *et al*. Pharmacokinetics and safety of CXA-101, a new antipseudomonal cephalosporin, in healthy adult male and female subjects receiving single- and multiple-dose intravenous infusions. *Antimicrob Agents Chemother* 2010; **54**: 3427–31.

11 LeBel M, Barbeau G, Vallee F *et al*. Pharmacokinetics of ceftazidime in elderly volunteers. *Antimicrob Agents Chemother* 1985; **28**: 713–5.

12 Sörgel F, Kinzig M. Pharmacokinetic characteristics of piperacillin/tazobactam. *Intensive Care Med* 1994; **20** Suppl 3: S14–20.

13 Bulik CC, Tessier PR, Keel RA *et al*. *In vivo* comparison of CXA-101 (FR264205) with and without tazobactam versus piperacillin/tazobactam using human simulated exposures against phenotypically diverse Gram-negative organisms. *Antimicrob Agents Chemother* 2012; **56**: 544–9.

14 Acred P. Therapeutic and kinetic properties of ceftazidime in animals. *Infection* 1983; **11** Suppl 1: S44–8.

15 dos Santos KV, Nicoli JR, Martins WA *et al*. Comparative activity of ertapenem and piperacillin–tazobactam in a murine systemic infection model with *Bacteroides fragilis* and *Escherichia coli*. *J Med Microbiol* 2007; **56**: 1576–9.

16 Roquilly A, Gautreau L, Segain JP *et al*. CpG-ODN and MPLA prevent mortality in a murine model of post-hemorrhage-*Staphylococcus aureus* pneumonia. *PLoS One* 2010; **5**: e13228.

17 Boutoille D, Marechal X, Pichenot M *et al*. FITC–albumin as a marker for assessment of endothelial permeability in mice: comparison with ^{125}I-albumin. *Exp Lung Res* 2009; **35**: 263–71.

18 Balamayooran G, Batra S, Fessler MB *et al*. Mechanisms of neutrophil accumulation in the lungs against bacteria. *Am J Respir Cell Mol Biol* 2010; **43**: 5–16.

19 Hsueh PR, Tseng SP, Teng LJ *et al*. Pan-drug-resistant *Pseudomonas aeruginosa* causing nosocomial infection at a university hospital in Taiwan. *Clin Microbiol Infect* 2005; **11**: 670–3.

20 Quale J, Bratu S, Gupta J *et al*. Interplay of efflux system, *ampC*, and *oprD* expression in carbapenem resistance of *Pseudomonas aeruginosa* clinical isolates. *Antimicrob Agents Chemother* 2006; **50**: 1633–41.

21 Juan C, Zamorano L, Pérez JL *et al*. Activity of a new antipseudomonal cephalosporin, CXA-101 (FR264205), against carbapenem-resistant and multidrug-resistant *Pseudomonas aeruginosa* clinical strains. *Antimicrob Agents Chemother* 2010; **54**: 846–51.

22 Moyá B, Zamorano L, Juan C *et al*. Activity of a new cephalosporin, CXA-101 (FR264205), against β-lactam-resistant *Pseudomonas aeruginosa* mutants selected *in vitro* and after antipseudomonal treatment of intensive care unit patients. *Antimicrob Agents Chemother* 2010; **54**: 1213–7.

23 Werner U, Szelenyi I. Measurement of MPO activity as model for detection of granulocyte infiltration in different tissues. *Inflamm Res* 1992; **36**: 101–3.

24 Mizgerd JP. Molecular mechanisms of neutrophil recruitment elicited by bacteria in the lungs. *Semin Immunol* 2002; **14**: 123–32.

25 Bianchi SM, Prince LR, McPhillips K *et al*. Impairment of apoptotic cell engulfment by pyocyanin, a toxic metabolite of *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med* 2008; **177**: 35–43.

26 Mizgerd JP. Acute lower respiratory tract infection. *N Engl J Med* 2008; **358**: 716–27.

27 Richman-Eisenstat J. Cytokine soup: making sense of inflammation in cystic fibrosis. *Pediatr Pulmonol* 1996; **21**: 3–5.

28 Saperstein S, Huyck H, Kimball E *et al*. The effects of interleukin-1β in tumor necrosis factor-α-induced acute pulmonary inflammation in mice. *Mediators Inflamm* 2009; **2009**: 958658.

29 Hilliard JJ, Melton JL, Hall L *et al*. Comparative effects of carbapenems on bacterial load and host immune response in a *Klebsiella pneumoniae* murine pneumonia model. *Antimicrob Agents Chemother* 2011; **55**: 836–44.

30 Mizgerd JP, Skerrett SJ. Animal models of human pneumonia. *Am J Physiol Lung Cell Mol Physiol* 2008; **294**: L387–98.

31 Garvy BA, Harmsen AG. The importance of neutrophils in resistance to pneumococcal pneumonia in adult and neonatal mice. *Inflammation* 1996; **20**: 499–512.

32 Hampton MB, Kettle AJ, Winterbourn CC. Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. *Blood* 1998; **92**: 3007–17.

33 Gaut JP, Byun J, Tran HD *et al*. Myeloperoxidase produces nitrating oxidants *in vivo*. *J Clin Invest* 2002; **109**: 1311–9.

34 Moyá B, Zamorano L, Juan C *et al*. Affinity of the new cephalosporin CXA-101 to penicillin-binding proteins of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2010; **54**: 3933–7.

35 Qiu H, KuoLee R, Harris G *et al*. High susceptibility to respiratory *Acinetobacter baumannii* infection in A/J mice is associated with a delay in early pulmonary recruitment of neutrophils. *Microbes Infect* 2009; **11**: 946–55.