International Journal of Antimicrobial Agents xxx (2008) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

In vivo efficacy of humanised intermittent versus continuous ceftazidime in combination with tobramycin in an experimental model of pseudomonal pneumonia

Delphine Croisier[a,b], Benoit Martha[a,b], Lionel Piroth[a,b], Pascal Chavanet[a,b,*]

[a] Infectious Diseases Department, University Hospital, Dijon, France
[b] LMI, EA 562 - LIMA, IFR Santé STIC, University of Burgundy, Dijon, France

ARTICLE INFO

Article history:
Received 10 June 2008
Accepted 11 July 2008

Keywords:
Pseudomonas infection
Ceftazidime
Continuous infusion
Tobramycin
Pharmacodynamics

ABSTRACT

In this study, we compared the efficacy of ceftazidime (CAZ) intermittent versus continuous infusion with or without tobramycin (TOB) for the treatment of pneumonia caused by *Pseudomonas aeruginosa* in rabbits. Treatments were humanised and mimicked intermittent CAZ (iCAZ) (2 g three times daily), continuous CAZ (cCAZ) (4 g once daily (qd)) and TOB (10 mg/kg qd). Minimum inhibitory concentrations (MICs) were 1 mg/L and 4 mg/L for TOB and CAZ, respectively. Bacterial efficacy in lungs was as follows: control, 9 ± 0.6 colony-forming units (CFU)/g; TOB monotherapy, 8 ± 0.5 CFU/g; iCAZ monotherapy, 7.8 ± 1.4 CFU/g; cCAZ monotherapy, 8 ± 0.4 CFU/g ($P = 0.005$); and iCAZ + TOB, 8 ± 0.5 CFU/g; cCAZ + TOB, 7.2 ± 0.3 CFU/g ($P < 0.05$). Bacterial efficacy in the spleen was as follows (% sterile): control, 4 ± 1.6 CFU/g (0%); TOB monotherapy, 1.7 ± 1.2 CFU/g (60%); iCAZ monotherapy, 3.5 ± 0.5 CFU/g (17%); cCAZ monotherapy, 1.5 ± 0.6 CFU/g (75%) ($P = 0.02$); and iCAZ + TOB, 2.1 ± 0.6 CFU/g (50%); cCAZ + TOB, 1.2 ± 0.3 CFU/g (82%) ($P < 0.05$). The time the drug concentration was above the MIC ($T >$ MIC) was 62% and 99% for iCAZ and cCAZ, respectively. We conclude that CAZ is more effective when administered continuously, especially for the sterilisation of septicaemia. A synergistic therapeutic effect of the association CAZ + TOB was observed in vivo, which can be explained by the longer $T >$ MIC of cCAZ. These findings suggest that continuous treatment with 4 g CAZ could be appropriate in patients with *P. aeruginosa* infections.

1. Introduction

Pseudomonas aeruginosa is a dangerous nosocomial pathogen [1,2] and infections can be difficult to treat because of resistance to many antibiotics, including those commonly used in hospitals.

There is still no consensus regarding the most effective way to administer parenteral β-lactam antibiotics for the treatment of bacterial infections. Today, continuous infusion of β-lactam drugs is often proposed since the pharmacodynamics is more effective [3–5]. However, in vivo experimental and clinical investigations failed to demonstrate a clear improvement in antibacterial effect.

The aim of this study was to compare the antibacterial efficacy of intermittent versus continuous ceftazidime (CAZ) in combination with tobramycin (TOB) on experimental pneumonia induced by *P. aeruginosa* in rabbits. In all of the experiments, treatments were humanised in order to simulate the human pharmacokinetics of the different treatments. These drugs were chosen since they are often recommended for treatment of pseudomonal infections [6–8].

2. Methods

2.1. Bacterial strain, growth conditions and antibiotics

Pseudomonas aeruginosa PA14 was used as the wild-type reference strain [9].

2.2. Minimum inhibitory concentration (MIC) determination

Drug MICs were determined by the standard dilution method in agar according to the Comité de l'Antibiogramme de la Société Française de Microbiologie [10].

2.3. Preparation of the inoculum

Before each animal experiment, one aliquot of *P. aeruginosa* PA14 was inoculated into Mueller–Hinton broth (MHB), cultured

* Corresponding author. Present address: Infectious Diseases Department, University Hospital, Hôpital du Bocage, BP 77908, 21000 Dijon, France. Tel.: +33 3 80 29 33 05; fax: +33 3 80 29 36 38.
E-mail address: pascal.chavanet@chu-dijon.fr (P. Chavanet).

0924-8579/$ – see front matter © 2008 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.
doi:10.1016/j.ijantimicag.2008.07.010

on agar plates and then incubated for 24 h at 37 °C. Three colonies were taken and incubated in 10 mL of MHB for 6 h at 37 °C, then cultured on agar plates for 18 h at 37 °C. This culture was then diluted in physiological saline to obtain a final concentration of 9.5 $\log_{10}$ colony-forming units (CFU)/mL. No adjuvant was used. This concentration was first determined using optical density measurements, in reference to a standard curve, and then confirmed by using successive dilution cultures.

2.4. Animals

Immunocompetent, male, New Zealand White rabbits (body weight 3 kg) were obtained from the Zootechnical Center (University of Burgundy, Dijon, France). They were placed in individual cages and were provided with food and water ad libitum.

2.5. Production of experimental P. aeruginosa pneumonia in rabbits

Central venous catheters were installed and pneumonia was induced as previously described [11,12]. Briefly, 24 h after jugular catheterisation, bacterial pneumonia was induced by endobronchial challenge with 0.5 mL of saline containing 9.5 $\log_{10}$ CFU/mL of one of the tested strains.

2.6. Human-like intravenous (i.v.) treatment with CAZ and TOB treatments

A 2-day treatment was delivered by infusion and started 5 h after bacterial challenge. CAZ and/or TOB were delivered over 2 days through the central venous catheter with changing infusion rates obtained by a computer-controlled electric pump in order to simulate the kinetics observed in human serum as previously described [11,12]. For CAZ, two types of administration were simulated in animals. (i) The human equivalent of intermittent CAZ (iCAZ), 2 g over a 0.5-h infusion three times daily [13–18]. For this treatment, the target concentration of serum CAZ was ca. 80 mg/L at the end of the infusion and a trough level of ca. 5 mg (the administered daily dose was 131 mg/kg/day). And (ii), the human equivalent of continuous infusion (cCAZ) at a daily dose of 4 g. For this method of delivery the target concentration of serum CAZ was ca. 30 mg/L [19,20] (the administered daily dose was 200 mg/kg/day).

TOB was administered to simulate in animals the human TOB treatment of 10 mg/kg i.v. once daily; the objective was to obtain a concentration of 30 mg/L at the end of the infusion [7,21].

2.7. Pharmacokinetic (PK) analysis

For each animal, the concentrations of antibiotics in serum were determined on iterative blood samples obtained through the second central catheter.

TOB and CAZ concentrations were determined by a disc plate bioassay with *Bacillus subtilis* ATCC 9466 and *Proteus mirabilis* ATCC 21100 as the indicator organisms, respectively. The limits of detection were 0.1 mg/L and 0.5 mg/L, respectively. Standard curves were established with solutions (progression from 0.5 mg/L to 7 mg/L) in serum. The linearity of the standard curves used for disc plate bioassays was at least 0.98 (r^2). The serum samples were diluted in serum to ensure that their concentrations would be within the range of those on the standard curve. The standard samples were assayed for each experiment and concentrations were assayed in duplicate. The between-day and within-day coefficients of variation for replicates were ≤10% and ≤12%, respectively, for both assays. In the case of drug combination, a cephalosporinase (P99; Sigma, Saint-Quentin-Fallavier, France) was used to inhibit CAZ, and polyethanol sulfonate was used to inhibit TOB, as previously described [10].

2.8. Evaluation of infection

Rabbits were anesthetised and sacrificed 1 h after the end of antibiotic infusion to avoid any carry-over effect. The spleen and each pulmonary lobe were weighed and homogenised in sterile water. Bacteria were counted in a sample of this crude homogenate by plating 10-fold dilutions on Mueller–Hinton agar and incubating the plates for 24 h at 37 °C; the threshold was 1 log CFU/g. Bacterial concentrations in each lung and in the spleen were determined after adjusting for weight. For each rabbit, the mean pulmonary bacterial concentration was calculated using the bacterial concentration of each lung (expressed as CFU/g).

The appearance of less susceptible *P. aeruginosa* was systematically looked for by plating lung homogenate on media containing 2–4× the MIC.

2.9. Pharmacodynamic (PD) analysis

The following parameters relating to MIC were calculated: peak drug concentration in serum (C_{max})/MIC ratio; area under the serum concentration–time curve from 0 h to 24 h (AUC_{0-24})/MIC ratio; and time the concentration was above the MIC (T > MIC) expressed as a percentage.

2.10. Statistical analysis

Results are expressed as the mean ± standard deviation. Quantitative variables were compared by analysis of variance (ANOVA), which was eventually completed by a post hoc analysis using the Bonferroni test. Non-parametric tests were also performed. Percentages were compared using the Fisher's exact test. A P-value of <0.05 was considered significant.

3. Results

MICs of *P. aeruginosa* PA14 were 1 mg/L and 4 mg/L for TOB and CAZ, respectively.

Simulation of human pharmacokinetics was successful in all of the animals (Fig. 1). C_{max} and trough drug concentration in serum (C_{min}), respectively, were 80 ± 20 mg/L and 2.6 ± 2 mg/L for iCAZ

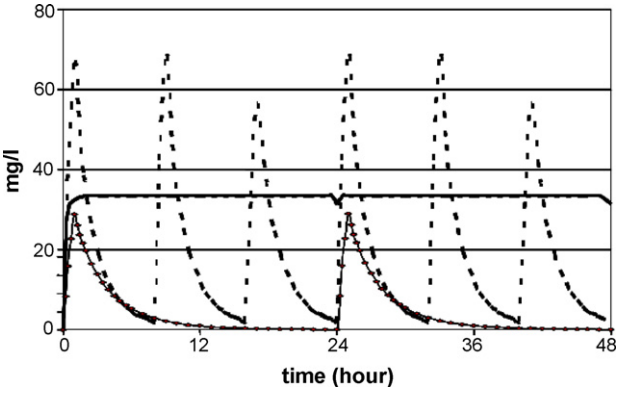

Fig. 1. Pharmacokinetics of human-like treatments with ceftazidime either in continuous infusion (cCAZ; —) or intermittent infusion (iCAZ; - - -) and with tobramycin (TOB; -♦-) in rabbits. iCAZ, cCAZ and TOB mimicked in animals the equivalent in humans of CAZ 2 g over 0.5 h three times daily, CAZ 4 g per day in continuous infusion and 10 mg/kg over 0.5 h once daily, respectively.

Please cite this article in press as: Croisier D, et al. In vivo efficacy of humanised intermittent versus continuous ceftazidime in combination with tobramycin in an experimental model of pseudomonal pneumonia. Int. J. Antimicrob. Agents (2008), doi:10.1016/j.ijantimicag.2008.07.010

Table 1
Antibacterial efficacy of human-like treatment with intermittent or continuous infusion ceftazidime (iCAZ and cCAZ, respectively) with or without human-like tobramycin (TOB) treatment on experimental pneumonia due to fully susceptible *Pseudomonas aeruginosa* PA14 in rabbits

	Control ($n=9$)	Monotherapy			Combination therapy	
		TOB ($n=5$)	iCAZ ($n=6$)	cCAZ ($n=4$)	iCAZ + TOB ($n=6$)	cCAZ + TOB ($n=11$)
Lung (log CFU/g)	9 ± 0.6[a]	8 ± 0.5	7.8 ± 1.4	8 ± 0.4	8 ± 0.5	7.2 ± 0.3[b]
Spleen (log CFU/g)	4 ± 1.6[c]	1.7 ± 1.2[d]	3.5 ± 0.5	1.5 ± 0.6[d]	2.1 ± 0.6	1.2 ± 0.3[d]
Sterilised spleen (n (%))	0[e]	3 (60)	1 (17)	3 (75)	3 (50)	9 (82)

CFU, colony-forming units.
[a] $P=0.005$ compared with treated groups.
[b] $P=0.05$ compared with other treated groups.
[c] No difference compared with iCAZ monotherapy, but significantly different from other treated groups ($P=0.02$).
[d] No difference.
[e] χ^2 for all groups, $P=0.01$. Significant ranking is as follows: control = iCAZ < TOB = iCAZ + TOB < cCAZ = cCAZ + TOB. Furthermore, intermittent infusions differed from continuous infusions ($P=0.01$) and combinations differed from monotherapies ($P=0.13$).

and 30 ± 8.6 mg/L and 0.5 ± 0.5 mg/L for TOB. The cCAZ serum levels were 31 ± 8 mg/L.

The AUC values for both CAZ treatments were equivalent (411 ± 210 and 410 ± 67 for iCAZ and cCAZ, respectively).

The results of antibacterial efficacy of the different regimens are shown in Table 1 and Fig. 2.

Overall, there was a contrast between the relatively weak efficacies in the lung for any regimen and the relatively high rate of sterilisation of blood cultures as measured by spleen cultures.

Compared with controls, all of the treatment regimens were associated with a weak but significant reduction in bacterial load in the lungs; however, only the combination of cCAZ and TOB was associated with a significant bacterial reduction compared with other treated groups.

In the spleen, no significant bacterial reduction was observed when animals were given iCAZ compared with non-treated animals. All other regimens were associated with a significant antibacterial effect. The most active regimens were cCAZ with or without TOB and TOB alone. When the proportion of splenic sterilisation was considered, again iCAZ was ineffective compared with controls. In contrast, the cCAZ regimens with or without TOB were the most active, reaching 80% sterilisation.

No bacteria with decreased susceptibility to either CAZ or TOB were found in the residual population of pulmonary or splenic bacteria.

The PD data related to the MIC are shown in Table 2.

Although overall exposure was almost the same for both CAZ regimens, as measured by AUC/MIC, their PK/PD profiles were different. The C_{min}/MIC ratio was as high as 7.8 for cCAZ and, as expected, almost zero for iCAZ. Furthermore, cCAZ was associated with antibiotic concentrations that were always above the MIC,

Table 2
Pharmacodynamics of human-like treatments with intermittent or continuous infusion ceftazidime (iCAZ and cCAZ, respectively) with or without human-like tobramycin (TOB) treatment on experimental pneumonia due to fully susceptible *Pseudomonas aeruginosa* PA14 in rabbits

	TOB	iCAZ	cCAZ
C_{max}/MIC	30.8 ± 8.6	20 ± 10[a]	7.8 ± 2[a]
C_{min}/MIC	–	0.7 ± 0.6[b]	7.8 ± 2[b]
AUC_{0-24}/MIC	101 ± 46	103 ± 52	102.7 ± 17
T>MIC (%)	45 ± 15	63 ± 31[c]	99 ± 1[c]

C_{max}, peak drug concentration in serum; MIC, minimum inhibitory concentration; C_{min}, trough drug concentration in serum; AUC_{0-24}, area under the serum concentration–time curve from 0 to 24 h; T > MIC, time the drug concentration was above the MIC.
[a] $P=0.001$.
[b] $P=0.001$.
[c] $P=0.009$.

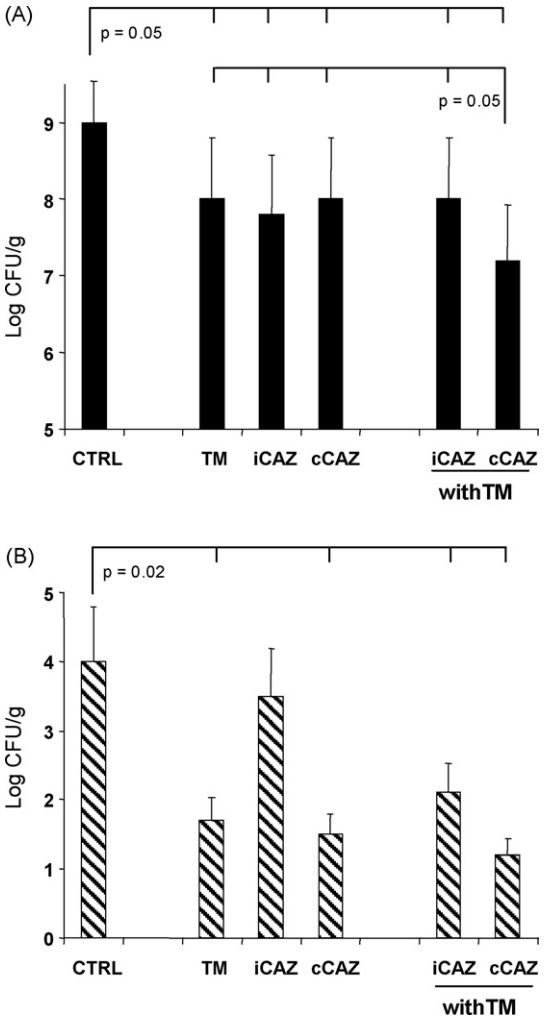

Fig. 2. Antibacterial efficacy of human-like treatments of intermittent or continuous ceftazidime (iCAZ and cCAZ, respectively) with or without human-like tobramycin treatment (TM) on experimental pneumonia caused by fully susceptible *Pseudomonas aeruginosa* PA14 in rabbits: (A) lungs; and (B) spleens. Results are expressed as mean of bacterial concentration (log colony-forming units (CFU)/g). Comparisons between groups were done using analysis of variance (ANOVA) and Bonferroni test if indicated. The proportions of spleen sterilisation were significantly different between groups (χ^2, $P=0.01$); significant ranking was as follows (no. sterile/no. of animals): control (0/9) = iCAZ (1/6) < TM (3/5) = iCAZ + TM (3/6) < cCAZ (3/4) = cCAZ + TM (9/11). Furthermore, intermittent infusions differed from continuous infusions ($P=0.01$) and combinations differed from monotherapies ($P=0.13$).

Please cite this article in press as: Croisier D, et al. In vivo efficacy of humanised intermittent versus continuous ceftazidime in combination with tobramycin in an experimental model of pseudomonal pneumonia. Int. J. Antimicrob. Agents (2008), doi:10.1016/j.ijantimicag.2008.07.010

whilst T > MIC for the iCAZ regimen was around two-thirds of the infusion interval.

For TOB, the mean C_{max}/MIC ratio was as high as 30.

4. Discussion

To our knowledge, this is the first study to investigate the efficacies of CAZ and TOB combinations using human-like treatments in experimental bacteraemic pseudomonal pneumonia. Furthermore, the model used [22] was very close to human pseudomonal pneumonia, especially with regard to bacterial concentration [23–31], and was also close to the human therapeutic situation since human-like pharmacokinetics was successfully achieved. In these conditions, we found that continuous infusion of CAZ with or without TOB was the most effective regimen. This is in complete concordance with the current concepts of pharmacodynamics [32,33] since cCAZ is associated with the highest T > MIC.

Like others [34,35], we found a synergistic effect of the combination CAZ and TOB in lungs in vivo, but it is noteworthy that this benefit was not observed when CAZ was infused intermittently every 8 h. Taking into account that both CAZ treatments were associated with the same AUC, the lack of increased antibacterial effect of this specific TOB + CAZ combination can be explained by the relatively short period of T > MIC when CAZ was infused intermittently. Indeed, even in the combination, the antipseudomonal activity was linked to the latter PK/PD parameter [36,37]. Thus, in these human-like conditions, this CAZ + TOB combination was associated with an additional or synergistic effect if T > MIC of CAZ is maximal throughout the continuous infusion.

These observations could also be related to the pulmonary disposition of CAZ in animals, which appears to be higher with continuous than with intermittent infusion [38], and to a longer half-life in the respiratory compartments than in serum [39]. Also, in animals the passage of TOB from the serum to lungs was found to be as high as 40–70% [38,40,41], whereas in humans the passage from serum to lung tissue is ca. 20% for CAZ [42] and even lower for TOB [43–46]. These facts may explain the relatively low efficacy of either regimen observed in our study, since humanised treatments were used.

Indeed, although significant, the bacterial reductions observed in the lungs of treated animals were low. This observation is not only in accordance with our previous work [22] but also with other studies in which the treatments were not humanised [47]. This finding is also close to the human situation in which antipseudomonal treatment is associated with a transient reduction in the bacterial concentration in the respiratory tract [29,48]. This lack of pseudomonal eradication explains the occurrence of resistance in the respiratory tract of patients [29,48–51], which is in accordance with in vitro findings [52,53]. However, in our study we failed to find any *Pseudomonas* isolates that were less susceptible. This finding may be related to the relatively short duration of treatment. Indeed, in clinical settings resistance occurred within the first week of antibiotic treatment [49,54].

The efficacy of continuous infusion of CAZ was high in the spleen, as measured both by bacterial reduction and by the proportion of sterilisation; the addition of TOB was not associated with increased efficacy. In contrast, iCAZ exhibited almost no antibacterial efficacy even though no mutants were found. However, the addition of TOB to iCAZ was associated with an improvement in efficacy compared with iCAZ alone but not when compared with TOB monotherapy.

In humans, the ability of a treatment to sterilise blood cultures is associated with a reduction in mortality in patients with pseudomonal infections [55–57]. In our study, no spontaneous death occurred in treated animals compared with 40% in untreated animals (P = 0.01). In this context, it is of interest to note that in our model the highest proportion of spleen culture sterilisation was obtained with cCAZ. Other investigators observed similar findings but only when the pseudomonal strain exhibited decreased susceptibility to CAZ [58]. Although the animal models were different (endocarditis versus pneumonia), taken together these results converge to strongly suggest that cCAZ is associated with a better in vivo antibacterial effect than iCAZ.

We conclude that for the treatment of pseudomonal infections, CAZ should be administered by continuous infusion even in combination with TOB.

Funding: Funding was obtained from the MEDEX Society.

Competing interests: None declared.

Ethical approval: The experiment was approved by the Ethical Committee for Animals Investigations of the University of Burgundy (No. 4405).

References

[1] Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, et al. Risk factors for antimicrobial resistance and influence of resistance on mortality in patients with bloodstream infection caused by *Pseudomonas aeruginosa*. Microb Drug Resist 2005;11:68–74.
[2] Vidal F, Mensa J, Almela M, Martinez JA, Marco F, Casals C, et al. Epidemiology and outcome of *Pseudomonas aeruginosa* bacteremia, with special emphasis on the influence of antibiotic treatment. Analysis of 189 episodes. Arch Intern Med 1996;156:2121–6.
[3] Kasiakou SK, Sermaides GJ, Michalopoulos A, Soteriades ES, Falagas ME. Continuous versus intermittent intravenous administration of antibiotics: a meta-analysis of randomised controlled trials. Lancet Infect Dis 2005;5:581–9.
[4] Roberts JA, Paratz J, Paratz E, Krueger WA, Lipman J. Continuous infusion of beta-lactam antibiotics in severe infections: a review of its role. Int J Antimicrob Agents 2007;30:11–8.
[5] Mouton JW, Vinks AA. Continuous infusion of beta-lactams. Curr Opin Crit Care 2007;13:598–606.
[6] Döring G, Conway SP, Heijerman HGM, Hodson ME, Hoiby N, Smyth A, et al. Antibiotic therapy against *Pseudomonas aeruginosa* in cystic fibrosis: a European consensus. Eur Respir J 2000;16:749–67.
[7] Smyth A, Tan KH, Hyman-Taylor P, Mulheran M, Lewis S, Stableforth D, et al. Once versus three-times daily regimens of tobramycin treatment for pulmonary exacerbations of cystic fibrosis—the TOPIC study: a randomised controlled trial. Lancet 2005;365:573–8.
[8] Masterton RG, Galloway A, French G, Street M, Armstrong J, Brown E, et al. Guidelines for the management of hospital-acquired pneumonia in the UK: report of the Working Party on Hospital-Acquired Pneumonia of the British Society for Antimicrobial Chemotherapy. J Antimicrob Chemother 2008;62:5–34.
[9] Rahme LG, Stevens EJ, Wolfort SF, Shao J, Tompkins RG, Ausubel FM. Common virulence factors for bacterial pathogenicity in plants and animals. Science 1995;268:1899–902.
[10] Comité de l'Antibiogramme de la Société Française de Microbiologie. Report of the Comité de l'Antibiogramme de la Société Française de Microbiologie. Technical recommendations for in vitro susceptibility testing. Clin Microbiol Infect 1996;251:11–25.
[11] Piroth L, Martin L, Coulon A, Lequeu C, Duong M, Buisson M, et al. Development of a new experimental model of penicillin-resistant *Streptococcus pneumoniae* pneumonia and amoxicillin treatment by reproducing human pharmacokinetics. Antimicrob Agents Chemother 1999;43:2484–92.
[12] Croisier D, Chavanet P, Lequeu C, Ahanou A, Nierlich A, Neuwirth C, et al. Efficacy and pharmacodynamics of simulated human-like treatment with levofloxacin on experimental pneumonia induced with penicillin-resistant pneumococci with varying susceptibilities to fluoroquinolones. J Antimicrob Chemother 2002;50:349–60.
[13] Drusano GL, Joshi J, Forrest A, Ruxer R, Standiford H, Leslie J, et al. Pharmacokinetics of ceftazidime, alone or in combination with piperacillin or tobramycin, in the sera of cancer patients. Antimicrob Agents Chemother 1985;27:605–7.
[14] LeBel M, Barbeau G, Vallee F, Bergeron MG. Pharmacokinetics of ceftazidime in elderly volunteers. Antimicrob Agents Chemother 1985;28:713–5.
[15] Ljungberg B, Nilsson-Ehle I. Influence of age on the pharmacokinetics of ceftazidime in acutely ill, adult patients. Eur J Clin Pharmacol 1988;34:173–8.
[16] Warns H, Lode H, Harnoss CM, Kemmerich B, Koeppe P, Wagner J. Multiple dose pharmacokinetics and therapeutic results with ceftazidime. J Antimicrob Chemother 1983;12(Suppl. A):235–40.
[17] Naber KG, Kees F, Grobecker H. Ceftazidime: pharmacokinetics in young volunteers versus elderly patients and therapeutic efficacy with complicated urinary tract infections. J Antimicrob Chemother 1983;12(Suppl. A):41–5.
[18] Leroy A, Leguy F, Borsa F, Spencer GR, Fillastre JP, Humbert G. Pharmacokinetics of ceftazidime in normal and uremic subjects. Antimicrob Agents Chemother 1984;25:638–42.

Please cite this article in press as: Croisier D, et al. In vivo efficacy of humanised intermittent versus continuous ceftazidime in combination with tobramycin in an experimental model of pseudomonal pneumonia. Int. J. Antimicrob. Agents (2008), doi:10.1016/j.ijantimicag.2008.07.010

[19] Mouton JW, Vinks AA, Punt NC. Pharmacokinetic–pharmacodynamic modeling of activity of ceftazidime during continuous and intermittent infusion. Antimicrob Agents Chemother 1997;41:733–8.
[20] Cousson J, Floch T, Vernet-Garnier V, Appriou M, Petit JS, Jovenin N, et al. Pharmacodynamic interest of ceftazidime continuous infusion vs intermittent bolus administration in patients with severe nosocomial pneumonia. Pathol Biol (Paris) 2005;53:546–50.
[21] Beringer PM, Vinks AATMM, Jelliffe RW, Shapiro BJ. Pharmacokinetics of tobramycin in adults with cystic fibrosis: implications for once-daily administration. Antimicrob Agents Chemother 2000;44:809–13.
[22] Martha B, Croisier D, Durand D, Hocquet D, Plesiat P, Piroth L, et al. In-vivo impact of the MexXY efflux system on aminoglycoside efficacy in an experimental model of *Pseudomonas aeruginosa* pneumonia treated with tobramycin. Clin Microbiol Infect 2006;12:426–32.
[23] Fetzer AE, Werner AS, Hagstrom JW. Pathologic features of pseudomonal pneumonia. Am Rev Respir Dis 1967;96:1121–30.
[24] Noone P, Rogers BT. Pneumonia caused by coliforms and *Pseudomonas aeruginosa*. J Clin Pathol 1976;29:652–6.
[25] Potts SB, Roggli VL, Spock A. Immunohistologic quantification of *Pseudomonas aeruginosa* in the tracheobronchial tree from patients with cystic fibrosis. Pediatr Pathol Lab Med 1995;15:707–21.
[26] Rose HD, Heckman MG, Unger JD. *Pseudomonas aeruginosa* pneumonia in adults. Am Rev Respir Dis 1973;107:416–22.
[27] Burns JL, Van Dalfsen JM, Shawar RM, Otto KL, Garber RL, Quan JM, et al. Effect of chronic intermittent administration of inhaled tobramycin on respiratory microbial flora in patients with cystic fibrosis. J Antimicrob Chemother 1999;179:1190–6.
[28] Lynch JP 3rd. Hospital-acquired pneumonia: risk factors, microbiology, and treatment. Chest 2001;119(2 Suppl):373S–84S.
[29] Crouch Brewer S, Wunderink RG, Jones CB, Leeper KV. Ventilator-associated pneumonia due to *Pseudomonas aeruginosa*. Chest 1996;109:1019–29.
[30] Garau J, Gomez L. *Pseudomonas aeruginosa* pneumonia. Curr Opin Infect Dis 2003;16:135–43.
[31] Rello J, Bodi M, Mariscal D, Navarro M, Diaz E, Gallego M, et al. Microbiological testing and outcome of patients with severe community-acquired pneumonia. Chest 2003;123:174–80.
[32] Drusano GL. Antimicrobial pharmacodynamics: critical interactions of 'bug and drug'. Nat Rev Microbiol 2004;2:289–300.
[33] Drusano GL. Pharmacokinetics and pharmacodynamics of antimicrobials. Clin Infect Dis 2007;45(Suppl. 1):S89–95.
[34] Gordin FM, Rusnak MG, Sande MA. Evaluation of combination chemotherapy in a lightly anesthetized animal model of *Pseudomonas* pneumonia. Antimicrob Agents Chemother 1987;31:398–403.
[35] Cappelletty DM, Kang SL, Palmer SM, Rybak MJ. Pharmacodynamics of ceftazidime administered as continuous infusion or intermittent bolus alone and in combination with single daily-dose amikacin against *Pseudomonas aeruginosa* in an in vitro infection model. Antimicrob Agents Chemother 1995;39:1797–801.
[36] den Hollander JG, Fuursted K, Verbrugh HA, Mouton JW. Duration and clinical relevance of postantibiotic effect in relation to the dosing interval. Antimicrob Agents Chemother 1998;42:749–54.
[37] Mouton JW, van Ogtrop ML, Andes D, Craig WA. Use of pharmacodynamic indices to predict efficacy of combination therapy in vivo. Antimicrob Agents Chemother 1999;43:2473–8.
[38] Girardi C, Tonnellier M, Goldstein I, Sartorius A, Wallet F, Rouby JJ. Lung deposition of continuous and intermittent intravenous ceftazidime in experimental *Pseudomonas aeruginosa* bronchopneumonia. Intensive Care Med 2006;32:2042–8.
[39] McColm AA, Ryan DM. Penetration of ceftazidime into the rabbit respiratory tract. J Antimicrob Chemother 1986;18:593–7.
[40] Eisenberg EJ, Conzentino P, Eickhoff WM, Cundy KC. Pharmacokinetic measurement of drugs in lung epithelial lining fluid by microdialysis: aminoglycoside antibiotics in rat bronchi. J Pharmacol Toxicol Methods 1993;29:93–8.
[41] Valcke Y, Pauwels R, Van der Straeten M. The penetration of aminoglycosides into the alveolar lining fluid of rats. The effect of airway inflammation. Am Rev Respir Dis 1990;142:1099–103.
[42] Boselli E, Breilh D, Rimmele T, Poupelin JC, Saux MC, Chassard D, et al. Plasma and lung concentrations of ceftazidime administered in continuous infusion to critically ill patients with severe nosocomial pneumonia. Intensive Care Med 2004.
[43] Boselli E, Breilh D, Djabarouti S, Guillaume C, Rimmele T, Gordien JB, et al. Reliability of mini-bronchoalveolar lavage for the measurement of epithelial lining fluid concentrations of tobramycin in critically ill patients. Intensive Care Med 2007;33:1519–23.
[44] Braude AC, Hornstein A, Klein M, Vas S, Rebuck AS. Pulmonary disposition of tobramycin. Am Rev Respir Dis 1983;127:563–5.
[45] Carcas AJ, Garcia-Satue JL, Zapater P, Frias-Iniesta J. Tobramycin penetration into epithelial lining fluid of patients with pneumonia. Clin Pharmacol Ther 1999;65:245–50.
[46] Mazzei T, Novelli A, De Lalla F, Mini E, Periti P. Tissue penetration and pulmonary disposition of tobramycin. J Chemother 1995;7:363–70.
[47] Pennington JE, Stone RM. Comparison of antibiotic regimens for treatment of experimental pneumonia due to *Pseudomonas*. J Infect Dis 1979;140:881–9.
[48] Rello J, Mariscal D, March F, Jubert P, Sanchez F, Valles J, et al. Recurrent *Pseudomonas aeruginosa* pneumonia in ventilated patients: relapse or reinfection? Am J Respir Crit Care Med 1998;157:912–6.
[49] Reinhardt A, Kohler T, Wood P, Rohner P, Dumas JL, Ricou B, et al. Development and persistence of antimicrobial resistance in *Pseudomonas aeruginosa*: a longitudinal observation in mechanically ventilated patients. Antimicrob Agents Chemother 2007;51:1341–50.
[50] Fish DN, Piscitelli SC, Danziger LH. Development of resistance during antimicrobial therapy: a review of antibiotic classes and patient characteristics in 173 studies. Pharmacotherapy 1995;15:279–91.
[51] Hocquet D, Berthelot P, Roussel-Delvallez M, Favre R, Jeannot K, Bajolet O, et al. *Pseudomonas aeruginosa* may accumulate drug resistance mechanisms without losing its ability to cause bloodstream infections. Antimicrob Agents Chemother 2007;51:3531–6.
[52] Henrichfreise B, Wiegand I, Luhmer-Becker I, Wiedemann B. Development of resistance in wild-type and hypermutable *Pseudomonas aeruginosa* strains exposed to clinical pharmacokinetic profiles of meropenem and ceftazidime simulated in vitro. Antimicrob Agents Chemother 2007;51:3642–9.
[53] Henrichfreise B, Wiegand I, Pfister W, Wiedemann B. Resistance mechanisms of multiresistant *Pseudomonas aeruginosa* from Germany and correlation with hypermutation. Antimicrob Agents Chemother 2007.
[54] Lodise TP, Miller CD, Graves J, Furuno JP, McGregor JC, Lomaestro B, et al. Clinical prediction tool to identify patients with *Pseudomonas aeruginosa* respiratory tract infections at greatest risk for multidrug resistance. Antimicrob Agents Chemother 2007;51:417–22.
[55] Kang C-I, Kim S-H, Kim H-B, Park S-W, Choe Y-J, Oh M-D, et al. *Pseudomonas aeruginosa* bacteremia: risk factors for mortality and influence of delayed receipt of effective antimicrobial therapy on clinical outcome. Clin Infect Dis 2003;37:745–51.
[56] Lodise Jr TP, Patel N, Kwa A, Graves J, Furuno JP, Graffunder E, et al. Predictors of 30-day mortality among patients with *Pseudomonas aeruginosa* bloodstream infections: impact of delayed appropriate antibiotic selection. Antimicrob Agents Chemother 2007;51:3510–5.
[57] Micek ST, Lloyd AE, Ritchie DJ, Reichley RM, Fraser VJ, Kollef MH. *Pseudomonas aeruginosa* bloodstream infection: importance of appropriate initial antimicrobial treatment. Antimicrob Agents Chemother 2005;49:1306–11.
[58] Robaux MA, Dube L, Caillon J, Bugnon D, Kergueris MF, Navas D, et al. In vivo efficacy of continuous infusion versus intermittent dosing of ceftazidime alone or in combination with amikacin relative to human kinetic profiles in a *Pseudomonas aeruginosa* rabbit endocarditis model. J Antimicrob Chemother 2001;47:617–22.

Please cite this article in press as: Croisier D, et al. In vivo efficacy of humanised intermittent versus continuous ceftazidime in combination with tobramycin in an experimental model of pseudomonal pneumonia. Int. J. Antimicrob. Agents (2008), doi:10.1016/j.ijantimicag.2008.07.010