

## Efficacy of pulsatile amoxicillin and clarithromycin dosing alone and in combination in a murine pneumococcal pneumonia model

Heather K. Sun<sup>1</sup>, Su Young Lee<sup>1</sup>, Mary Anne Banevicius<sup>1</sup>, Xiaoli Du<sup>1</sup>,  
Dana Maglio<sup>1</sup> and David P. Nicolau<sup>1,2\*</sup>

<sup>1</sup>Center for Anti-Infective Research and Development, Hartford Hospital, 80 Seymour Street, Hartford, CT 06102, USA; <sup>2</sup>Division of Infectious Diseases, Hartford Hospital, Hartford, CT 06102, USA

Received 1 March 2005; returned 11 June 2005; revised 13 June 2005; accepted 25 June 2005

**Objectives:** Amoxicillin and clarithromycin have been proven to be effective in the treatment of community-acquired pneumonia. This study investigated the *in vivo* bactericidal efficacy of a novel, pulsatile dosing strategy for amoxicillin and clarithromycin, when used as monotherapy and combination therapy.

**Methods:** A neutropenic murine pneumonia model was used to assess the bactericidal activity of amoxicillin and clarithromycin, when the same total daily dose was administered as a traditional regimen (every 8 h and every 12 h, respectively) or as a pulsatile regimen (four doses of antibiotic given every 2 h over the first 6 h of the day) against three isolates of *Streptococcus pneumoniae* of varying resistance profiles. The three isolates consisted of SP21 (macrolide and penicillin susceptible), SP100 [*mef*(A) gene], and SP107 [*mef*(A) + *erm*(B) genes].

**Results:** Pulsatile dosing showed similar reductions in bacterial density for amoxicillin and clarithromycin when either drug was given alone compared with traditional dosing regimens against all three bacterial isolates. When amoxicillin and clarithromycin were combined, improved activity was found compared with monotherapy. Overall, when comparing the different combination regimens, the pulsatile regimens provided similar activity compared with the traditional regimens. For one isolate, SP107, pulsatile amoxicillin combination regimens were less effective compared with traditionally dosed amoxicillin combination regimens.

**Conclusions:** Pulsatile dosing resulted in comparable bactericidal activity against the three isolates tested and may represent an alternative dosing strategy, which may help to alleviate problems with patient adherence to drug therapy.

Keywords: *Streptococcus pneumoniae*, pulsatile dosing, mouse model

### Introduction

*Streptococcus pneumoniae* is the leading causative pathogen of community-acquired respiratory tract infections, such as community-acquired pneumonia (CAP), otitis media and sinusitis.<sup>1</sup> Amoxicillin and clarithromycin are two potent antibiotics that have been relied upon heavily in the treatment of pneumococcal infections. Their efficacy has been proven in many investigations from experimental infection models to clinical trials,<sup>2–7</sup> and both agents have been incorporated into practice guidelines for empirical therapy in the treatment of CAP.<sup>8,9</sup> Combination therapy

using a macrolide plus a  $\beta$ -lactam has been shown to reduce in-hospital mortality in patients with bacteraemic pneumococcal pneumonia.<sup>10</sup>

Rates of penicillin and macrolide-resistant *S. pneumoniae* have continued to rise at an alarming rate. Macrolide and penicillin resistance has been shown to be ~30% in the USA.<sup>11</sup> As emergence of resistance in *S. pneumoniae* continues to grow, new treatment options and strategies are warranted.

This study evaluated Advancis Pharmaceuticals' novel dosing strategies as they undergo formulation of a drug delivery system. These new strategies utilize pulsatile dosing, which produces short

\*Correspondence address. Center for Anti-Infective Research and Development, Hartford Hospital, 80 Seymour Street, Hartford, CT 06102, USA. Tel: +1-860-545-3940; Fax: +1-860-545-3992; E-mail: dnicola@harthosp.org

bursts of drug early in the dosing interval followed by a prolonged dose-free period, which could potentially reduce the development of resistance against the particular antimicrobial agent.<sup>12</sup> Additionally, with the ability to control the release of drug into the body, these new dosing strategies may allow for once-daily dosing of antibiotic regimens, which could increase patient adherence to medication.

Pulsatile dosing has been shown in *in vitro* studies to be better or as effective as traditional dosing regimens for certain antibiotics.<sup>12–14</sup> Previously, a study was conducted utilizing an *in vitro* model, which assessed the efficacy of clarithromycin alone and in combination with amoxicillin against *S. pneumoniae*.<sup>14</sup> The study investigators found that the clarithromycin and amoxicillin combination pulsatile regimens yielded significant bactericidal activity. Although results from *in vitro* studies are valuable, *in vivo* studies must be conducted to determine whether the effectiveness of the treatment strategies can be translated to animals and ultimately to humans. Our current study evaluated the *in vivo* efficacy of pulsatile dosing of amoxicillin and clarithromycin alone and in combination against *S. pneumoniae* in a neutropenic murine pneumonia model.

## Materials and methods

### Antimicrobial agents and bacteria

Amoxicillin analytical grade powder (Sigma Laboratories, St Louis, MO, USA) was used for all *in vitro* testing. Commercially available amoxicillin for oral suspension, USP (STADA Pharmaceuticals, Inc., Cranbury, NJ, USA) was used for all *in vivo* testing and prepared according to the package insert. Clarithromycin analytical grade powder (Abbott Laboratories, North Chicago, IL, USA) was used for all *in vitro* and *in vivo* testing. For animal dosing with clarithromycin, 95% ethanol and sterile 0.1 M phosphate buffer (pH 6.5) in a 1:10 dilution was used as the vehicle, and the clarithromycin suspension was sonicated for 30 min prior to dosing.

Three clinical isolates of *S. pneumoniae* were utilized in this study. One isolate was macrolide/penicillin susceptible (SP21), one isolate contained the *mef(A)* gene (SP100) and one isolate contained the *mef(A)* and *erm(B)* gene (SP107). All strains were stored in skimmed milk medium (Becton Dickinson, Cockeysville, MD, USA) at  $-80^{\circ}\text{C}$  and subcultured twice onto Trypticase soy agar with 5% sheep blood (Becton Dickinson) before use in all *in vitro* and *in vivo* experiments.

### *In vitro* susceptibility tests

The median MICs of amoxicillin and clarithromycin were determined for each isolate in triplicate using standard NCCLS methodology for broth microdilution.<sup>15</sup>

### Lung infection model

Specific, pathogen-free, female ICR mice ( $\sim 25$  g) were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN, USA). Hartford Hospital's (Hartford, CT, USA) Institutional Animal Care and Use Committee reviewed and approved the methodology for use of these animals. Mice were rendered neutropenic by intraperitoneal injection of cyclophosphamide 150 mg/kg at 4 days and 100 mg/kg 1 day prior to inoculation.<sup>16</sup> All animals were maintained and utilized in accordance with recommendations from the National Research Council and were provided food and water *ad libitum*.

A suspension of *S. pneumoniae* was prepared from the second subculture of bacteria, which was incubated for  $<20$  h. The inoculum was then adjusted to a 3.0 McFarland turbidity standard in a 5%

dextrose saline solution to  $\sim 10^8$  cfu/mL. The bacterial density of each inoculum was confirmed by serial dilution and plating of each suspension.

The animals were lightly anaesthetized with isoflurane (2% v/v in 100% oxygen carrier) until the respiratory rate was reduced upon visual inspection to one breath per second. Infection was induced by oral instillation of 0.05 mL of bacterial suspension with simultaneous blockage of the nares to cause bacterial aspiration into the lungs. Mice recovered fully in an oxygen-enriched chamber before randomization into control and treatment groups. Treatment with active drug regimens and controls began 12–14 h post-inoculation (0 h dosing time).

### Pharmacokinetic studies

Pharmacokinetic studies were conducted for the commercially prepared amoxicillin for oral suspension, USP product. Neutropenic ICR mice were prepared as described above for the lung infection model and infected with SP100. Single doses of amoxicillin suspension (12.5, 25 and 100 mg/kg) were administered orally to mice 12–14 h after inoculation. Blood samples were collected from six to 12 mice by intracardiac puncture at 8–10 time points per regimen over 24 h. After blood collection, samples were centrifuged and the serum was collected and stored at  $-80^{\circ}\text{C}$  until assayed. In addition, bronchial alveolar lavage (BAL) samples were obtained simultaneously to blood collection at 0.5 and 1.5 h for determination of drug levels in epithelial lining fluid (ELF). BAL samples from six mice per time point were obtained by cannulating the exposed trachea with a 22 gauge catheter and flushing with 0.4 mL of saline four times. The recovered volume of BAL fluid was centrifuged and stored at  $-80^{\circ}\text{C}$  until assayed. ELF volume was calculated using the urea dilution method.<sup>17</sup>

Concentrations of amoxicillin in murine serum and BAL were determined with a validated HPLC procedure. The serum assay was linear over a range of 0.2–20 mg/L ( $r^2 = 1.0$ ). Intraday coefficients of variation for the low (0.5 mg/L) and high (15 mg/L) quality control samples were 1.78 and 3.48%, respectively. Interday coefficients of variation for the quality control samples were 2.34 and 5.11%, respectively. Pharmacokinetic analyses for the individual amoxicillin serum concentration profiles were performed using a one-compartment model (WinNonlin, version 3.3, Pharsight Corp., Mountain View, CA, USA) and a weighting scheme of  $1/\text{yhat}^2$ . Pharmacokinetic studies for total drug concentrations of clarithromycin were previously completed at our institution and were not repeated here.<sup>18</sup>

### Treatment regimens

Twelve to 14 h after bacterial inoculation, groups of six mice were administered amoxicillin and clarithromycin as monotherapy or as combination therapy in a 0.2 mL volume via oral gavage. Mice receiving combination therapy received 0.2 mL of amoxicillin, followed immediately by 0.2 mL of clarithromycin. For traditional dosing, drug administration took place every 8 h for amoxicillin and every 12 h for clarithromycin. For pulsatile dosing, the same total daily dose of either amoxicillin or clarithromycin was used, but the dose was divided into four individual doses given every 2 h over the first 6 h.

This study was completed in three phases for each bacterial isolate. The first phase consisted of dose–response experiments to discover the total daily amoxicillin dose given traditionally that would yield an  $\sim 1$  log decrease in bacterial density compared with the 0 h controls. The second phase involved taking this same total daily dose of amoxicillin, and two other doses, and comparing changes in bacterial density between traditional dosing and pulsatile dosing. The third phase of the study consisted of assessing differences in bactericidal activity between clarithromycin monotherapy when administered traditionally or in a pulsatile fashion. For clarithromycin, the total daily dose

## Amoxicillin and clarithromycin dosing

used was the human simulated dose (300 mg/kg) as determined from previous studies.<sup>18</sup> In addition, efficacy of combination therapy with the amoxicillin dose producing 1 log kill and the human simulated dose of clarithromycin was assessed when given as both traditional dosing as well as pulsatile dosing.

### Efficacy as assessed by bacterial density

Twelve to 14 h after the infection was established and simultaneous to initiation of dosing (0 h), control mice (six per group) were sacrificed. After 24 h of treatment, vehicle-treated controls and treatment groups were sacrificed (six per group). Animals were euthanized by CO<sub>2</sub> inhalation followed by cervical dislocation. Lungs were aseptically removed and individually homogenized in 1 mL of normal saline. Serial dilutions were plated on Trypticase soy agar with 5% sheep blood for cfu determinations. Efficacy (change in bacterial density) was calculated by subtracting the mean log<sub>10</sub> cfu per lung of the 0 h control mice sacrificed just prior to dosing from the mean log<sub>10</sub> cfu per lung of untreated controls and treatment groups at the end of 24 h of therapy.

### Data analysis

For efficacy assessments, a sample size calculation was applied, which considered the fact that optimal dosing regimens for typical antimicrobial agents usually produce an ~2–3 log<sub>10</sub> decrease in bacterial density with a %CV of 40. In order to have an observed mean that deviates from the true mean by no more than one standard deviation using a two-sided, 95% confidence interval with 80% probability, six data points are required; thus six animals were included in each group to ensure statistical viability. Change in bacterial density in lungs for amoxicillin-treated and placebo-treated control animals was reported using descriptive statistics. An inhibitory sigmoid E<sub>max</sub> dose–effect model derived from the Hill equation was used to characterize the relationship between amoxicillin dose and efficacy. Spearman's correlation coefficient test evaluated the degree to which change in bacterial density correlated with dose.

## Results

Median MIC values for amoxicillin and clarithromycin against the three test isolates were determined in triplicate according to the NCCLS microdilution broth technique. These three isolates were chosen based on their varied macrolide resistance genotypes. MIC results and genotypic descriptions of all isolates are found in Table 1.

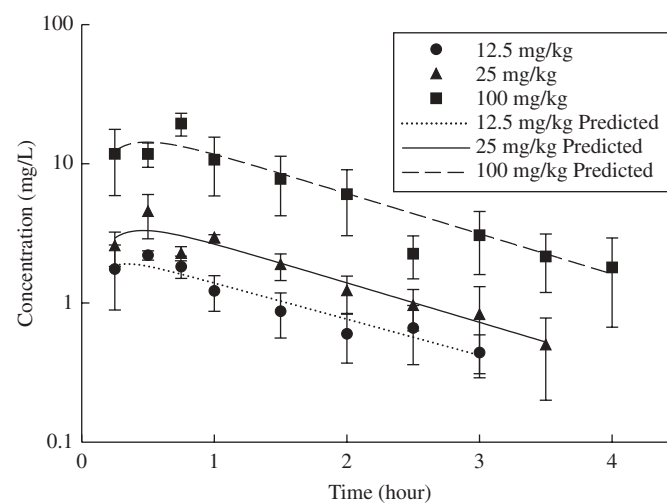
Pharmacokinetic parameters in neutropenic mice were determined for amoxicillin oral suspension, USP, based on single-dose pharmacokinetic testing of amoxicillin 12.5, 25 and 100 mg/kg using a one-compartment model. The pharmacokinetic total drug concentration versus time profiles for all three doses are displayed in Figure 1. Symbols represent actual mean data points for each regimen. Solid and broken lines represent predicted profiles for each regimen. AUC values (3, 6 and 30 mg·h/L for the 12.5, 25 and 100 mg/kg doses, respectively) and C<sub>max</sub> values (2, 4, 14 mg/L for the 12.5, 25 and 100 mg/kg doses, respectively) were dose proportional, whereas t<sub>1/2</sub> remained consistent for all three doses. The final pharmacokinetic parameters for amoxicillin oral suspension are found in Table 2 and reported as mean values with standard deviation.

ELF concentrations of amoxicillin were calculated based on drug concentrations found in mouse BAL fluid and are displayed in Table 3, along with serum concentrations at simultaneous time points. Percent penetration into ELF at both 0.5 and 1.5 h

**Table 1.** Median MIC values for all test isolates

Isolate <sup>a</sup>	MIC (mg/L)		Genotype
	amoxicillin	clarithromycin	
SP21	0.03	0.03	non- <i>mef(A)</i> , non- <i>erm(B)</i>
SP100	2.0	1.0	<i>mef(A)</i>
SP107	2.0	2.0	<i>mef(A)</i> + <i>erm(B)</i>

<sup>a</sup>Internal strain designation.



**Figure 1.** Pharmacokinetic profile of amoxicillin oral suspension, USP.

**Table 2.** Pharmacokinetic parameters for amoxicillin oral suspension, USP

Parameter	Mean	SD
V (L/kg)	5.32	0.26
K <sub>a</sub> (h <sup>-1</sup> )	5.97	1.94
K <sub>e</sub> (h <sup>-1</sup> )	0.64	0.04
t <sub>1/2</sub> (h)	1.09	0.06
CL (L/h/kg)	3.40	0.23

**Table 3.** Amoxicillin serum and calculated ELF concentrations from mouse BAL fluid at time 0.5 and 1.5 h

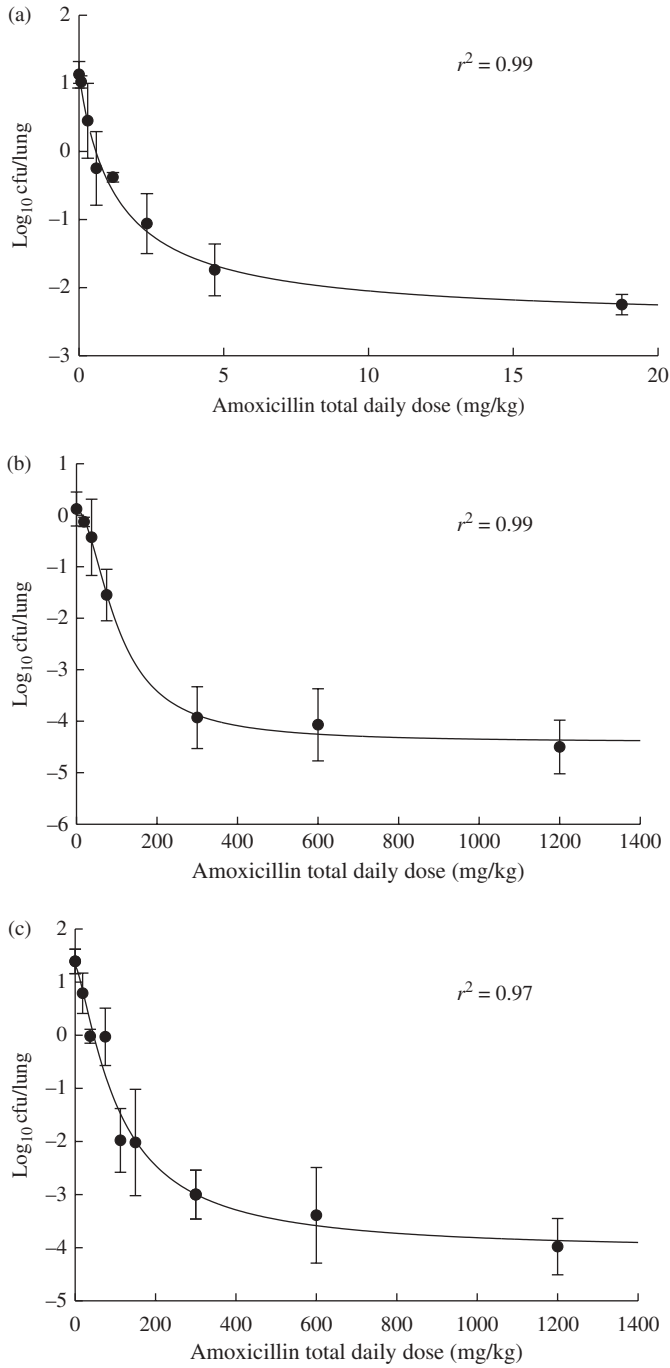
Time (h)	Dose (mg/kg)	Mean (SD) concentrations (mg/L)		Penetration (%)
		serum	ELF	
0.5	12.5	1.37 (0.29)	2.20 (0.17)	62
	25	2.30 (0.53)	4.45 (1.56)	52
	100	5.37 (1.58)	11.76 (2.34)	46
1.5	12.5	0.42 (0.14)	0.87 (0.31)	48
	25	1.12 (0.39)	1.85 (0.40)	61
	100	4.30 (0.55)	7.78 (3.55)	55

ranged from 46–62% for all doses, with a mean (SD) penetration of 54% (7%).

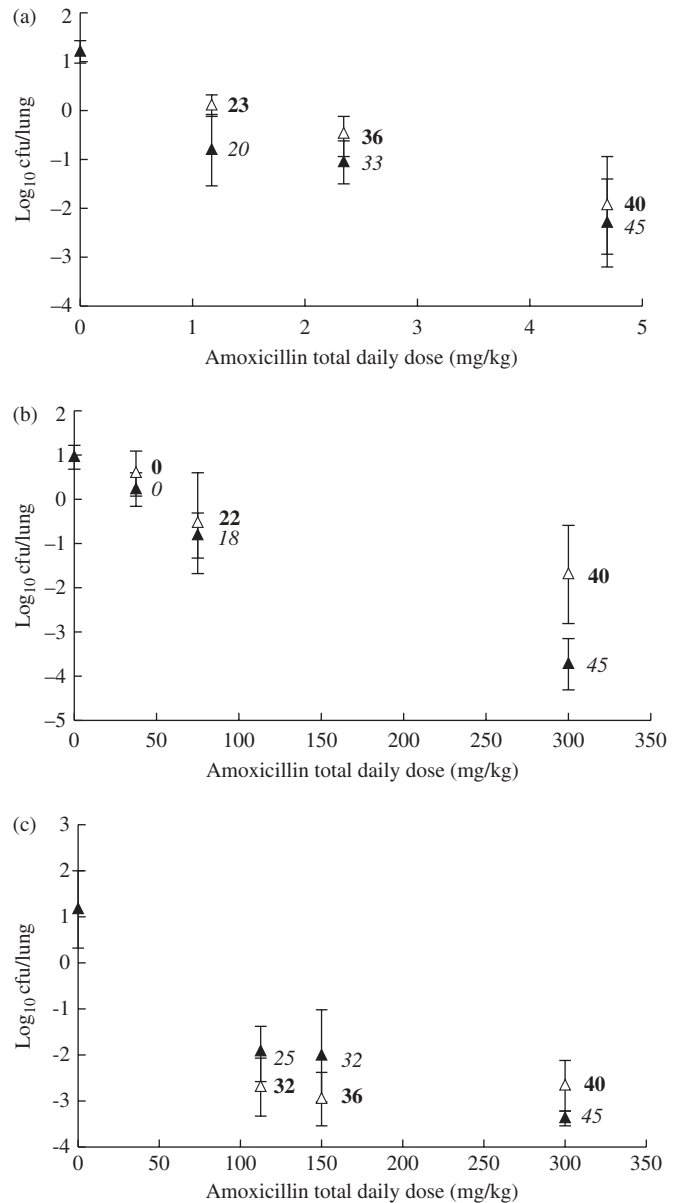
Excellent recovery of all *S. pneumoniae* isolates was obtained within infected lungs of mice. At initiation of treatment, bacterial densities ranged from 5.51–7.8 log<sub>10</sub> cfu/lung (mean 6.94 ± 0.7). Bacterial density increased over 24 h in untreated controls by a mean of 1.02 ± 0.41 log<sub>10</sub> cfu/lung. Dose–response curves with amoxicillin for all three isolates are found in Figure 2. Dose corresponded well with changes in bacterial density ( $r^2$  values

of 0.97–0.99). The purpose of the dose–response experiments for amoxicillin was to determine the total daily dose, when given every 8 h, that would yield ~1 log kill against the three different strains of *S. pneumoniae* compared with the 0 h controls. Those doses were determined to be 2.34 mg/kg for SP21, 75 mg/kg for SP100 and 112.5 mg/kg for SP107.

When comparing pulsed amoxicillin dosing and traditional thrice-daily dosing, changes in bacterial density as well as total drug, percentage time above the MIC of the pathogen (%t > MIC) exposure were similar among the three isolates tested (see Figure 3). For SP100, the 300 mg/kg total daily dose, given as a pulsatile regimen, resulted in less bacterial kill compared with the traditional, every 8 h, regimen.



**Figure 2.** Dose–response curves after 24 h of orally dosed amoxicillin administered as traditional dosing (every 8 h) to neutropenic ICR mice infected with: (a) SP21; (b) SP100; and (c) SP107.



**Figure 3.** Effect comparisons of amoxicillin administered as traditional dosing (filled triangles) versus pulsatile dosing (open triangles) in neutropenic ICR mice infected with: (a) SP21; (b) SP100; and (c) SP107. %t > MIC exposure is represented by the number to the right of each data point.

## Amoxicillin and clarithromycin dosing

**Table 4.** Mean (SD) changes in bacterial density and %t > MIC of amoxicillin (AMX) and clarithromycin (CLR) for all antimicrobial regimens against all three test isolates

Isolate	Antimicrobial regimen (doses in mg/kg)	Log <sub>10</sub> change cfu/lung	AMX %t > MIC	CLR %t > MIC
SP21	24 h control	1.25 (0.15*)	0	0
	0.78 AMX every 8 h	-1.06 (0.44)	33	0
	0.59 AMX pulsed	-0.53 (0.41)	36	0
	150 CLR twice a day	-1.54 (0.64)	0	100
	75 CLR pulsed	-1.70 (0.45)	0	100
	0.78 AMX every 8 h + 150 CLR twice a day	-2.06 (0.11)	33	100
	0.59 AMX pulsed + 150 CLR twice a day	-2.11 (0.08)	36	100
	0.78 AMX every 8 h + 75 CLR pulsed	-2.23 (0.21)	33	100
	0.59 AMX pulsed + 75 CLR pulsed	-1.87 (0.30)	36	100
SP100	24 h control	0.68 (0.41*)	0	0
	25 AMX every 8 h	-0.82 (0.51)	18	0
	18.75 AMX pulsed	-0.54 (1.14)	22	0
	150 CLR twice a day	0.41 (0.75)	0	45
	75 CLR pulsed	0.47 (0.40)	0	60
	25 AMX every 8 h + 150 CLR twice a day	-1.80 (1.48)	18	45
	18.75 AMX pulsed + 150 CLR twice a day	-0.36 (0.26)	22	45
	25 AMX every 8 h + 75 CLR pulsed	-1.37 (0.41)	18	60
	18.75 AMX pulsed + 75 CLR pulsed	-1.04 (0.65)	22	60
SP107	24 h control	1.06 (0.39*)	0	0
	37.5 AMX every 8 h	-1.98 (0.60)	25	0
	28.13 AMX pulsed	-2.70 (0.63)	32	0
	150 CLR twice a day	-0.26 (0.44)	0	22
	75 CLR pulsed	0.04 (0.64)	0	31
	37.5 AMX every 8 h + 150 CLR twice a day	-3.42 (0.78 <sup>a,b</sup> )	25	22
	28.13 AMX pulsed + 150 CLR twice a day	-1.60 (0.57 <sup>b</sup> )	32	31
	37.5 AMX every 8 h + 75 CLR pulsed	-2.84 (0.38 <sup>c</sup> )	25	22
28.13 AMX pulsed + 75 CLR pulsed	-1.02 (0.69 <sup>a,c</sup> )	32	31	

\*Value in parentheses represents the standard error of the mean.

<sup>a</sup>P = <0.001.

<sup>b</sup>P = 0.004.

<sup>c</sup>P = 0.005.

Clarithromycin monotherapy using the human simulated dose of 150 mg/kg every 12 h was tested and compared against the same total daily dose given as a pulsed dose. Change in bacterial density for clarithromycin alone was similar to previous experiments performed at our institution.<sup>18</sup> For SP21, the mean change in log<sub>10</sub> cfu/lung compared with the 0 h control was -1.54 log<sub>10</sub> cfu/lung for the traditional twice-daily regimen and -1.70 log<sub>10</sub> cfu/lung for the pulsatile regimen. For SP100, the mean change in log<sub>10</sub> cfu/lung was 0.41 for the traditional regimen versus 0.47 for the pulsatile regimen. For SP107, the mean change in log<sub>10</sub> cfu/lung for the traditional regimen and the pulsatile regimen was -0.26 and 0.04, respectively.

Amoxicillin and clarithromycin were used in the following combinations: amoxicillin every 8 h plus clarithromycin every 12 h, amoxicillin pulsed dosing plus clarithromycin every 12 h, amoxicillin every 8 h plus clarithromycin pulsed dosing and amoxicillin pulsed dosing plus clarithromycin pulsed dosing. Table 4 displays the mean change in log<sub>10</sub> cfu/lung at 24 h, as well the total drug %t > MIC exposure of amoxicillin and clarithromycin, for all dosing regimens (monotherapy and combination therapy) against the three *S. pneumoniae* isolates. Since neither amoxicillin nor clarithromycin are highly protein bound, ~20<sup>19</sup> and 50%,<sup>20</sup> respectively, total drug exposure was presented here.

Overall, the amoxicillin and clarithromycin combination regimens showed increased kill compared with amoxicillin and clarithromycin given alone. Against SP21 and SP100, the pulsed amoxicillin and pulsed clarithromycin combination regimens provided similar efficacy compared with the other combination regimens (*P* value = 0.12 and 0.09, respectively). For SP107, traditionally dosed amoxicillin regimens appeared to improve the bactericidal activity compared with the pulsatile amoxicillin combination regimens, regardless of whether it was combined with traditionally dosed or pulsatile dosed clarithromycin.

### Discussion

Amoxicillin and clarithromycin have been used for years in the successful treatment of pneumococcal infections such as community-acquired pneumonia. However, bacterial resistance continues to rise steadily causing reduced efficacy in antimicrobials that used to be highly effective. Resistance has the potential to lead to treatment failures, increased morbidity, increased costs and even death.<sup>21</sup> In addition to the continued need for the development of new antimicrobial agents, a need also exists for the development and evaluation of new treatment strategies that aim to enhance pharmacodynamic effects and decrease resistance. We used a

neutropenic murine pneumonia model to investigate the comparative effects of a novel pulsatile regimen to that of traditional dosing regimens with amoxicillin and clarithromycin when both agents are given either as monotherapy or combination therapy.

This was the first *in vivo* study assessing the efficacy of pulsatile dosing regimens. Several *in vitro* studies have been performed already with varying antimicrobial agents. Ibrahim *et al.*<sup>12</sup> used an *in vitro* model to compare the activity of pulse-dosed metronidazole with traditional thrice-daily regimens against *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*. They found bactericidal activity among the different regimens to be comparable.

Similarly Cha *et al.*<sup>13</sup> used an *in vitro* model to compare the pharmacodynamics of amoxicillin simulated as a pulsatile dose and as traditional, multiple doses against *S. pneumoniae*. Overall they found bacterial density reductions between treatment regimens to be similar for the susceptible isolate they used and greater with pulsatile dosing against the less susceptible strain.

In our analysis, bactericidal activity was similar among the three traditional versus pulsatile doses for amoxicillin alone. For the susceptible isolate, SP21, %t > MIC exposure was similar for the three total daily doses tested. There was slightly less exposure at the highest total daily amoxicillin dose, but bactericidal activity was still the same between the traditional dosing and pulsatile dosing groups. For the two macrolide-resistant isolates, slightly better %t > MIC exposure was observed for the lower doses. Although reduction in bacterial density for either isolate at the lower doses was comparable, the mean change in log<sub>10</sub> cfu/lung at the lower doses was slightly more for the pulsatile regimen against SP107. At 300 mg/kg/day of amoxicillin, %t > MIC exposure was lower in the pulsatile regimens compared with the traditional regimens. At this high dose with SP107, bactericidal activity was similar, but for SP100, there was less bacterial kill, reflecting the decrease in %t > MIC exposure at this dose.

Leuthner *et al.*<sup>14</sup> used an *in vitro* model to compare activities of clarithromycin alone and in combination with amoxicillin when simulated as traditional dosing and pulsatile dosing. They used two isolates, one of which was susceptible to both clarithromycin and amoxicillin and another that was resistant to clarithromycin and intermediately resistant to amoxicillin. Results of the study revealed clarithromycin pulsatile dosing to be more effective in eradicating bacteria at higher doses. The investigators also found pulsed combination therapy with amoxicillin and clarithromycin to be more effective against the resistant isolate and equally efficacious against the susceptible isolate.

In our study, changes in log<sub>10</sub> cfu/lung for clarithromycin monotherapy were similar for the traditional and pulsatile regimens against all *S. pneumoniae* isolates. For the amoxicillin and clarithromycin combination regimens, we found changes in bacterial density to be greater compared with clarithromycin monotherapy, which was consistent with the Leuthner study. In comparing the combination regimens with each other, bactericidal activity was similar among all combination regimens for the susceptible isolate, which is also consistent with the results of Leuthner *et al.* However, we did not find superior activity *in vivo* with the pulsed amoxicillin and pulsed clarithromycin regimen compared with the other combination regimens for the two macrolide-resistant isolates. For SP100, all combination regimens were similar with no significant differences in mean change in log<sub>10</sub> cfu/lung. For SP107, there was variability among the different combination regimens. Regimens with amoxicillin dosed traditionally had greater kill compared with the pulsed amoxicillin regimens.

Our current *in vivo* study reveals the comparable bactericidal activity of pulsatile regimens compared with traditional dosing regimens of amoxicillin and clarithromycin as monotherapy and as combination therapy. Compared with the *in vitro* studies that have been performed to date, our *in vivo* results in infected neutropenic mice do not show any superior bactericidal activity with amoxicillin and clarithromycin as monotherapy or combination therapy when given as traditional versus pulsatile dosing. Since %t > MIC exposure for both amoxicillin and clarithromycin were similar among the traditional dosing groups versus the pulsatile dosing groups, one would expect the bactericidal efficacies between the two dosing schemes to be similar. Our results showed that efficacies were indeed similar among the traditional dosing groups and pulsatile dosing groups, thus indicating that exposure was the key driver to bactericidal efficacy.

While pulsatile antibiotic administration did not appear to improve bactericidal activity of either amoxicillin or clarithromycin, since it provided similar %t > MIC exposure for both agents, it did not deter from the activity provided from traditional dosing. Therefore, this technique of pulsatile dosing may allow for ease of dosing and improved patient adherence. We did not assess the ability for pulsatile administration to suppress resistance, but this issue should be examined in the future. Other additional *in vivo* studies are warranted to further evaluate the benefits of pulsed dosing and offer more insight into its value in clinical practice.

## Acknowledgements

This study was supported by a research grant from Advancis Pharmaceutical Corporation (Germantown, MD, USA). We thank Toral Patel, Vesley Martell, Lindsay Tuttle and Deborah Santini for their assistance in completing this study.

## References

1. Felmingham D, Reinert RR, Hirakata Y *et al.* Increasing prevalence of antimicrobial resistance among isolates of *Streptococcus pneumoniae* from the PROTEKT surveillance study, and comparative *in vitro* activity of the ketolide, telithromycin. *J Antimicrob Chemother* 2002; **50** Suppl. S1: 25–37.
2. Azoulay-Dupuis E, Moine P, Bedos P *et al.* Amoxicillin dose-effect relationship with *Streptococcus pneumoniae* in a mouse pneumonia model and roles of *in vitro* penicillin susceptibilities, autolysis, and tolerance properties of the strains. *Antimicrob Agents Chemother* 1996; **40**: 941–6.
3. Woodnutt G, Berry V. Efficacy of high-dose amoxicillin-clavulanate against experimental respiratory tract infections caused by strains of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 1999; **43**: 35–40.
4. Maglio D, Capitano B, Banevicius MA *et al.* Efficacy of clarithromycin against *Streptococcus pneumoniae* expressing *mef(A)*-mediated resistance. *Int J Antimicrob Agents* 2004; **23**: 498–501.
5. Maglio D, Capitano B, Banevicius MA *et al.* Differential efficacy of clarithromycin in lung versus thigh infection models. *Chemotherapy* 2004; **50**: 63–6.
6. Hoffman HL, Klepser ME, Ernst EJ *et al.* Influence of macrolide susceptibility on efficacies of clarithromycin and azithromycin against *Streptococcus pneumoniae* in a murine lung infection model. *Antimicrob Agents Chemother* 2003; **47**: 739–46.
7. Gotfried MH, Dattani D, Riffer E *et al.* A controlled, double-blind, multicenter study comparing clarithromycin extended-release tablets and

## Amoxicillin and clarithromycin dosing

levofloxacin tablets in the treatment of community-acquired pneumonia. *Clin Ther* 2002; **24**: 736–51.

8. Mandell LA, Bartlett JG, Dowell SF *et al*. Update of practice guidelines for the management of community-acquired pneumonia in immunocompetent adults. *Clin Infect Dis* 2003; **37**: 1405–33.

9. Heffelfinger JD, Dowell SF, Jorgensen JH *et al*. Management of community-acquired pneumonia in the era of pneumococcal resistance: a report from the drug-resistant *Streptococcus pneumoniae* therapeutic working group. *Arch Intern Med* 2000; **160**: 1399–1408.

10. Martinez JA, Horcajada JP, Almela M *et al*. Addition of a macrolide to a  $\beta$ -lactam-based empirical antibiotic regimen is associated with lower in-hospital mortality for patients with bacteremic pneumococcal pneumonia. *Clin Infect Dis* 2003; **36**: 389–95.

11. Sun H, Maglio D, Nicolau D. Macrolide resistance in *Streptococcus pneumoniae*: Mechanisms, patterns, and clinical implications of resistance. *Connecticut Med* 2004; **68**: 571–76.

12. Ibrahim KH, Gunderson BW, Hermesen ED *et al*. Pharmacodynamics of pulse dosing versus standard dosing: In vitro metronidazole activity against *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*. *Antimicrob Agents Chemother* 2004; **48**: 4195–99.

13. Cha R, Rybak MJ. Pulsatile delivery of amoxicillin against *Streptococcus pneumoniae*. *J Antimicrob Chemother* 2004; **54**: 1067–71.

14. Leuthner KD, Cheung CM, Rybak MJ. Pulsatile delivery of clarithromycin alone and in combination with amoxicillin against *Streptococcus pneumoniae* (SPN) with reduced susceptibility to these agents.

In *Programs and Abstracts of the Forty-fourth, Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, USA*. Abstract A1169. American Society for Microbiology, Washington, DC, USA.

15. National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests For Bacteria that Grow Aerobically—Fourteenth Edition: Approved Standard*. NCCLS, Wayne, PA, USA.

16. Joly-Guillou ML, Wolff M, Pocard JJ *et al*. (1997) Use of a new mouse model of *Acinetobacter baumannii* pneumonia to evaluate the post-antibiotic effect of imipenem. *Antimicrob Agents Chemother* 2004; **41**: 345–51.

17. Conte JE, Golden JA, Duncan S *et al*. Intrapulmonary pharmacokinetics of clarithromycin and of erythromycin. *Antimicrob Agents Chemother* 1995; **39**: 334–38.

18. Tessier PR, Kim M, Zhou W *et al*. Pharmacodynamic assessment of clarithromycin in a murine model of pneumococcal pneumonia. *Antimicrob Agents Chemother* 2002; **46**: 1425–34.

19. Amoxil [Prescribing Information]. Research Triangle Park, NC, USA: GlaxoSmithKline; 2004.

20. Biaxin [Prescribing Information]. North Chicago, IL, USA: Abbott Laboratories; 2003.

21. Hoban D, Waites K, Felmingham D. Antimicrobial susceptibility of community-acquired respiratory tract pathogens in North America in 1999–2000: findings of the PROTEKT surveillance study. *Diagn Microbiol Infect Dis* 2003; **45**: 251–9.