Enhanced efficacy of single-dose versus multi-dose azithromycin regimens in preclinical infection models

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Objectives: As a result of the prolonged half-life and unique pharmacokinetic and pharmacodynamic (PK-PD) characteristics of azithromycin, shorter dosing regimens are being evaluated for the treatment of community-acquired infections. To provide further support for a shorter dosing regimen, the efficacy of azithromycin was determined in preclinical infection models comparing single- versus multi-dose regimens.

Methods: The efficacy of single versus multi-dose regimens of azithromycin was compared in mouse pneumonia, acute peritonitis, and neutropenic thigh infection models and in a gerbil model of Haemophilus influenzae acute otitis media. Azithromycin was administered as a single oral dose on the first treatment day, or as two divided doses over 2 treatment days, or as three divided doses over 3 treatment days. The pharmacokinetics of azithromycin was profiled following single and multi-dose regimens with the single dose data fit to an $E_{\text{max}}$ model to characterize the PK-PD of azithromycin.

Results: In the mouse efficacy models, administration of single-dose azithromycin produced superior rates of survival and bacterial clearance compared with the same total dose divided over 2 or 3 days. In the gerbil model, a single dose sterilized the middle ear and more rapidly cleared $H$. influenzae. The pharmacokinetic evaluation confirmed similar total exposure (AUC) in serum and pulmonary tissue for the three regimens. Correlation of PK-PD parameters and antimicrobial efficacy confirmed a concentration-dependent and dosing-independent relationship for azithromycin.

Conclusions: These data are consistent with data reported from clinical studies and indicate that a single-dose regimen would be at least as effective as the same dose administered over several days.

Keywords: azithromycin, clarithromycin, amoxicillin/clavulanate, single-dose regimen, acute otitis media, Haemophilus influenzae, Streptococcus pneumoniae

Introduction

Azithromycin is an azalide antibiotic that has been in use for more than a decade in the treatment of community-acquired infections including acute otitis media (AOM) in infants and children. Although early labelling of oral azithromycin for paediatric indications mimicked the 5 day dosing regimens of oral $\beta$-lactam agents and the 10 day regimens of oral penicillin and cephalosporins, the recent adoption of 3 day and single-dose oral regimens for acute otitis media reflects a move to optimize drug efficacy. In this regard, recent studies have demonstrated that in uncomplicated AOM, single-dose oral azithromycin is as effective as 3 day oral azithromycin, single-dose intramuscular ceftriaxone, 10 day oral amoxicillin/clavulanate and 10 day high-dose amoxicillin. Compared with the 10 day amoxicillin/clavulanate regimen, single-dose azithromycin was associated with significantly better compliance.

The antimicrobial efficacy of a drug is determined by the inter-relationship between its pharmacokinetic and pharmacodynamic (PK-PD) properties. The development of an effective short-course or single-dose regimen requires a concentration-dependent antimicrobial effect; as drug concentration increases, the rate and extent of killing also increase, making the goal one of dosing to maximize drug concentration. For such agents, the PK-PD predictor of efficacy is $\text{AUC/MIC or } C_{\text{max}}/\text{MIC}$. In contrast, drugs such as clarithromycin and $\beta$-lactams display minimal concentration-dependent killing; the extent of killing is dependent on the duration of exposure and these drugs therefore require frequent dosing to maintain the drug concentration above the MIC for much of the dosing interval.
The pharmacokinetic properties that make azithromycin suitable for single-dose therapy are high tissue penetration, including high concentrations in the middle ear, and an extended elimination half-life of more than 60 h that allows for once daily dosing.\cite{8,9} Pharmacodynamic properties of azithromycin include bactericidal activity against key respiratory tract pathogens and a prolonged post-antibiotic or persistent effect.\cite{3} In addition, azithromycin is concentrated within phagocytes, which provide targeted delivery to the site of infection, further enhancing local tissue concentrations and improving in vivo efficacy.\cite{8,10,11} Furthermore, the PK-PD parameter that most closely correlates with the efficacy of azithromycin is AUC/MIC.\cite{12,13}

The objective of this study was to evaluate the impact of administering oral azithromycin as a single-dose regimen in several preclinical infection models and thus provide a scientific rationale in support of existing clinical efficacy data. In vivo efficacy studies were conducted with azithromycin administered orally, either as a single large dose or divided over 2 or 3 days in mouse models of acute peritonitis, neutropenic thigh infection, and pneumococcal pneumonia; and in a gerbil model of Haemophilus influenzae AOM. Additionally, serum and pulmonary tissue pharmacokinetics were established for the three dosing intervals and 24 h PK-PD parameters were correlated with efficacy. In this study, we demonstrate that azithromycin administered over a shorter (1 day) dosing period provides superior efficacy when compared with 2 or 3 day regimens. Most significantly, a 1 day regimen results in more rapid eradication of *H. influenzae* in the gerbil model of otitis media than does a regimen lasting several days.

**Materials and methods**

**Antibiotics**

Stock solutions of azithromycin (Zithromax; Pfizer), clarithromycin (Abbott Laboratories) and amoxicillin/clavulanate (GlaxoSmithKline) were prepared before each experiment and diluted in a 0.5% methyl cellulose or ethanol/Tween 80/phosphate-buffered saline (5:5:90, by volume) vehicle to the desired concentration.

**Bacterial strains**

Bacterial strains used in this study were: *Streptococcus pneumoniae* 02J1016, serotype 3, a penicillin- and macrolide-susceptible strain originally isolated from blood culture (strain P 4241); *Streptococcus pyogenes* 02C203, ATCC 12384, group A, type 3, a macrolide-susceptible strain; *Enterococcus faecalis* 03A1085, a vancomycin-susceptible, clinically derived strain; *H. influenzae* 54A1100, ATCC 43095, a non-serotype B, penicillin and macrolide-susceptible strain; *H. influenzae* 54A1128, a non-serotype B, penicillin-resistant and macrolide-susceptible strain (both *H. influenzae* strains were isolated from otitis media).

**MIC determination**

MICs for *S. pneumoniae*, *S. pyogenes*, *E. faecalis* and *H. influenzae* were determined using the broth microdilution procedure recommended by the NCCLS.\cite{14} Test trays were incubated at 35°C without CO₂. For testing streptococci, the cation-adjusted Mueller–Hinton broth was supplemented with 2–3% lyzed horse blood. For testing *H. influenzae*, freshly prepared Haemophilus Test Medium broth was used. MICs were determined a minimum of five times and modal MIC values were reported.

**Animals**

Female Swiss CF-1 mice (18–20 g) aged 5–6 weeks were used for pharmacokinetic studies and for *S. pneumoniae* and *S. pyogenes* infection; female DBA/2 mice (18–20 g) aged 5–6 weeks were used for *E. faecalis* and *H. influenzae* infection; female Mongolian gerbils (45–50 g) aged 6–7 weeks were used for *H. influenzae* infection. All animals were obtained from Charles River Laboratories, Inc. (Wilmington, MA, USA). All procedures involving animals were approved by, and were in compliance with guidelines established by, the Pfizer Institutional Animal Care and Use Committee.

**Pharmacokinetic evaluation**

For single dose pharmacokinetic evaluation of azithromycin, CF-1 mice were administered an oral dose of azithromycin at 200, 100, 50, 25 or 12.5 mg/kg. Blood samples were taken starting at 0.25 h post-dose and at pre-determined intervals over a 24 h period (5 mice per time point for a total of 30 mice per dose level). To evaluate the pharmacokinetics of accelerated dosing with azithromycin, CF-1 mice were orally administered azithromycin at 100 mg/kg once daily for 1 day (5 mice per time point for a total of 40 mice), 50 mg/kg once daily for 2 days (5 mice per time point for a total of 65 mice), or 33 mg/kg once daily for 3 days (5 mice per time point for a total of 105 mice). Blood and pulmonary tissue samples were taken starting at 0.5 h post-dose and at pre-determined intervals over a 96 h period. For all pharmacokinetic experiments, serum and lung tissue samples were prepared and maintained at −70°C until further analysis. Serum and pulmonary tissue concentrations of azithromycin were determined by a validated LC/MS assay using Turbo IonSpray mass spectrometry detection.\cite{15} The lower and upper limits of quantification for the assay were 50 μg/L and 5 mg/L, respectively, and intra- and inter-assay variability was <7%. Pharmacokinetic parameters were calculated using non-compartmental methods using WinNonlin 2.1 software (Pharsight Corporation, Mountain View, CA, USA). Single dose pharmacokinetic data were subsequently used to estimate 24 h pharmacodynamic parameters of AUC/MIC, Cₘₐₓ/MIC and time above MIC in order to explore the relationship between PK-PD parameters and antimicrobial effect (corrected for free fraction). Accelerated dosing pharmacokinetic data were used to establish the effect of dosing over 1, 2 or 3 days on serum and pulmonary tissue concentrations of azithromycin.

**PK-PD versus efficacy evaluation**

To evaluate the relationship between PK-PD parameters and antimicrobial efficacy, pulmonary (10 mice per dose level for a total of 180 mice per model), neutropenic thigh (5 mice per dose level for a total of 90 mice per model), and acute peritonitis infection models (10 mice per dose level for a total of 180 mice per model) were used in a total of five efficacy trials: normal CF-1 mice were challenged with a log phase culture of *S. pneumoniae* via the intranasal route (~10⁵ cfu/mouse in 40 μL); neutropenic CF-1 mice (neutropenia was induced using oral cyclophosphamide; 150 mg/kg 4 days prior and 100 mg/kg 1 day prior to infection) were challenged intramuscularly via the thigh with ~10⁵ cfu/mouse of either *S. pneumoniae* or *S. pyogenes* in 50 μL of vehicle; and normal CF-1 mice were challenged intraperitoneally with either *S. pneumoniae* (~10⁵ cfu/mouse in 500 μL) or *S. pyogenes* (~10⁵ cfu/mouse in 500 μL).

Oral azithromycin therapy was initiated 18 h after intranasal challenge, or 1.0 h after intraperitoneal or intramuscular challenge using a once per 24 h, once per 12 h, once per 6 h, or once per 3 h dosing interval that covered a 64-fold dose range for each infection model (0.39–200 mg/kg per day) and continued for a total of 24 h. Streptococcal clearance (efficacy) was determined in the pulmonary infection...
was scored over 6 days at which time the effective dose 50 (ED50) was determined using non-linear regression techniques with GraphPad Prism v4.0.

For the AOM model, gerbils were infected with $10^{3–4}$ cfu of *H. influenzae* via intra-bulla instillation. Oral therapy with azithromycin, clarithromycin, or amoxicillin/clavulanate (3.1–200, 6.2–400 and 3.1–200 mg/kg, respectively) was initiated 18 h after challenge and administered once daily for 1, 2 or 3 days as before (5 gerbils per dose level, total of 50 gerbils per study and done in triplicate). Bullae were tapped 72 h after initiation of therapy, washed with 100 µL of saline and recoverable *H. influenzae* were enumerated to a detection limit of 100 cfu. ED50 values were calculated from the percentage of animals that cleared the *H. influenzae* infection over the evaluated dose range at 72 h. For some experiments, oral therapy was initiated 24 h after intra-bulla challenge of gerbils and consisted of a total therapeutic dose of 200 mg/kg azithromycin administered once daily over 1, 2, or 3 days (15 gerbils per dosing regimen, 65 gerbils per study and repeated in triplicate). Bullae were tapped at 24, 48, 72 and 96 h following challenge, washed with 100 µL of saline and recoverable *H. influenzae* were enumerated to a detection limit of 100 cfu.

### Data analysis

A sigmoidal $E_{\text{max}}$ dose–response model derived from the Hill equation (four-parameter logistic equation with variable slope using GraphPad Prism v4.0) was used to determine the relationships between individual PK-PD parameters and antimicrobial efficacy outcomes of bacterial clearance and survival; $Y = D + (A - D)/(1 + 10^{(X-C)/D})$. For the four-parameter logistic equation, $Y$ is the observed effect, $D$ is the bottom, $A$ is the top, $C$ is the EC50 or 50% of the observed maximum effect, $X$ is the log of the concentration and $b$ is the Hill slope.

From these relationships, the coefficient of determination ($R^2$) value was calculated using non-linear least-squares multivariate regression analysis and subsequently used to estimate the goodness of fit for each PK-PD parameter. Non-linear regression analysis was also used to estimate ED50 values from 6 and 10 day survival data. Significance between dosing regimen outcomes for bacterial burden was determined using ANOVA (one-way) and when the F statistic reached significance ($P < 0.05$) then post-hoc comparison was made using Tukey’s multiple comparison test.

### Results

#### Pharmacokinetics of single- versus multi-dose azithromycin regimens

The systemic exposure of azithromycin increased in an approximately dose-proportional manner (Figure 1). Serum $C_{\text{max}}$ values ranged from 0.6 to 15 mg/L and AUC0–24 values ranged from 3 to 44 mg·h/L. Time to $C_{\text{max}}$ ($T_{\text{max}}$) ranged from 1 to 2 h. $C_{\text{max}}$ was highest when azithromycin was administered as a single dose of 100 mg/kg on 1 treatment day (100 mg/kg per day), rather than as a divided dose over 2 (50 mg/kg per day), or 3 treatment days (33.3 mg/kg per day) (Figure 2). Whereas $C_{\text{max}}$ was reached 2 h after administration of the single dose regimen, $C_{\text{max}}$ was delayed until 26 h when azithromycin was given as either two or three divided doses over a longer treatment period. Azithromycin displayed similar pharmacokinetic behaviour in pulmonary tissue, with $C_{\text{max}}$ again favouring early delivery of the entire azithromycin dose (Table 1).

#### Azithromycin single dose PK-PD versus efficacy findings

Composite data were obtained from five efficacy trials that measured bacterial burden and survival in mice infected with
S. pneumoniae or S. pyogenes and subsequently treated for 24 h with azithromycin. The relationship between efficacy [bacterial clearance and mouse survival expressed as efficacy (response)] and PK-PD parameters of AUC/MIC, $C_{\text{max}}$/MIC and time above MIC is shown in Figure 3. For each of the PK-PD parameters, there was a sigmoidal relationship with efficacy. The global PD parameter that best correlated with efficacy outcome was AUC/MIC with an $R^2$ value of 0.70, followed by $C_{\text{max}}$/MIC with an $R^2$ value of 0.52. A poor correlation was observed with time above MIC ($R^2 = 0.29$).

Efficacy of single- versus multi-dose azithromycin regimens

In the murine acute peritonitis infection model, a 1 day dosing regimen of oral azithromycin had a 50% protective effect on mouse 6 day survival at a significantly lower dose than a 2 and/or 3 day dosing regimen when S. pneumoniae, S. pyogenes, E. faecalis, or H. influenzae were used as the challenge organism (Table 2). In contrast, clarithromycin was much less active than 

**Table 1.** Comparison of azithromycin pharmacokinetic parameters in serum and pulmonary tissue following administration of a total dose of 100 mg/kg, given once daily over 1, 2 or 3 days

<table>
<thead>
<tr>
<th>Total dose (mg/kg)</th>
<th>Days of dosing</th>
<th>Individual dose (mg/kg per dose)</th>
<th>$C_{\text{max}}$ (mg/L)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>AUC$_{0-24}$ (mg·h/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum PK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>100</td>
<td>2.1</td>
<td>2</td>
<td>18.8</td>
</tr>
<tr>
<td>100</td>
<td>2</td>
<td>50</td>
<td>1.3</td>
<td>26</td>
<td>15.9</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>33.3</td>
<td>0.98</td>
<td>26</td>
<td>19.8</td>
</tr>
<tr>
<td>Pulmonary PK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>100</td>
<td>40</td>
<td>4</td>
<td>970</td>
</tr>
<tr>
<td>100</td>
<td>2</td>
<td>50</td>
<td>31</td>
<td>28</td>
<td>870</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>33.3</td>
<td>28</td>
<td>12</td>
<td>1048</td>
</tr>
</tbody>
</table>
azithromycin against *S. pneumoniae*, *S. pyogenes* and *H. influenzae* irrespective of which dosing regimen was used. Clarithromycin did show superior ED$_{50}$ activity against *E. faecalis* compared with azithromycin and was effective against this organism when administered as a 1 day dosing regimen. However, in general there was no clear relationship between clarithromycin activity and dosing regimen, since the 2 and 3 day regimens were optimal versus *S. pyogenes* while the 1 day regimen was superior for *E. faecalis*.

Similar results were obtained using a mouse pneumococcal pulmonary infection model, which used 10 day survival as an efficacy outcome measure. Table 3 shows an ED$_{50}$ value of 20 mg/kg (16–24 mg/kg) when azithromycin was administered as a single dose on 1 treatment day, compared with values of 27 mg/kg (23–32 mg/kg), and 49 mg/kg (28–71 mg/kg) when azithromycin was administered as a divided dose over 2 or 3 treatment days. In contrast, clarithromycin was poorly active irrespective of dosing regimen.

In the gerbil AOM model, the efficacy outcome of bacterial clearance at day 4 after challenge was not significantly different for therapy lasting 1, 2 or 3 days; however, when bacterial clearance was investigated with respect to time in the same model, significant differences were observed (Table 4 and Figure 4). When azithromycin was administered as a single dose of 200 mg/kg, bacterial burden at 48 h post-challenge (24 h post-dose) dropped to below the threshold for detection ($P < 0.001$ relative to control) and there was no re-growth at 72–96 h post-challenge ($P < 0.001$ relative to control for all time points). In contrast, maximal reduction of bacterial burden in animals administered a 2 or 3 day azithromycin regimen did not occur until 72 h post-challenge ($P \leq 0.001$ relative to control).

### Table 2. Effect of dosing regimen on the therapeutic activity of azithromycin and clarithromycin in a murine peritonitis model

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (mg/L)</th>
<th>1 day therapy</th>
<th>2 day therapy</th>
<th>3 day therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pyogenes</em> 02C0203</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>azithromycin</td>
<td>0.03</td>
<td>0.78$^a$ (0.71–1.00)</td>
<td>1.10 (1.02–1.35)</td>
<td>1.25 (1.18–1.91)</td>
</tr>
<tr>
<td>clarithromycin</td>
<td>0.03</td>
<td>11.7$^a$ (4.3–16.2)</td>
<td>2.5 (1.8–3.9)</td>
<td>3.8 (3.8–3.9)</td>
</tr>
<tr>
<td><em>Streptococcus pneumonia</em> 02J1016</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>azithromycin</td>
<td>0.10</td>
<td>8.7$^a$ (0.4–9.0)</td>
<td>7.6 (7.3–7.8)</td>
<td>15.1 (13.4–16.7)</td>
</tr>
<tr>
<td>clarithromycin</td>
<td>0.05</td>
<td>99.8 (64.4–118)</td>
<td>103.0 (72.2–169)</td>
<td>87.5 (70.5–92.4)</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> 03A1085</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>azithromycin</td>
<td>6.25</td>
<td>12.7$^a$ (11.5–13.6)</td>
<td>44.5 (6.0–147)</td>
<td>44.4 (14.1–55.2)</td>
</tr>
<tr>
<td>clarithromycin</td>
<td>3.12</td>
<td>6.1 (5.5–8.5)</td>
<td>16.6 (8.6–18.2)</td>
<td>23.1 (14.5–108)</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em> 54A1100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>azithromycin</td>
<td>0.5</td>
<td>30.3$^a$ (11.9–52.0)</td>
<td>48.0 (16.3–96.4)</td>
<td>147 (32.0–346)</td>
</tr>
<tr>
<td>clarithromycin</td>
<td>4</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

$^a$Indicates that 1 day therapy ED$_{50}$ value is significantly different from 2 and/or 3 day therapies.

### Table 3. Influence of dosing regimen on the therapeutic activity of azithromycin and clarithromycin in a murine pneumococcal pneumonia model

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (mg/L)</th>
<th>1 day therapy</th>
<th>2 day therapy</th>
<th>3 day therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pneumonia</em> 02J1016</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>azithromycin</td>
<td>0.10</td>
<td>20$^a$ (16–24)</td>
<td>27 (23–32)</td>
<td>49 (28–71)</td>
</tr>
<tr>
<td>clarithromycin</td>
<td>0.05</td>
<td>270$^a$ (259–297)</td>
<td>327 (262–389)</td>
<td>338 (297–430)</td>
</tr>
</tbody>
</table>

$^a$Indicates that 1 day therapy ED$_{50}$ value is significantly different from 2 and/or 3 day therapies.

### Discussion

Azithromycin has unique pharmacokinetic properties that result from the cellular accumulation of the drug and its subsequent slow, sustained release into the bloodstream, thereby allowing the drug to be administered in shortened regimens while maintaining therapeutic efficacy over a wide range of infections. An understanding of a particular drug’s PK-PD profile is critical for the rational development of dosing regimens that maximize therapeutic efficacy. In addition to improved clinical and bacteriological outcomes, adoption of a dosing regimen based on the PK-PD profile of a drug may assist in minimizing selective pressures for the development of antimicrobial resistance. Patient compliance, which is linked to clinical and bacteriological outcomes as well as to antimicrobial resistance, is also an important consideration and would be expected to be inherently high with an abbreviated regimen, leading to a reduced need for re-treatment.
There is existing clinical support for single-dose azithromycin in the treatment of community-acquired infections, coming from clinical trials of AOM and also pharmacokinetic data from healthy adult volunteers showing equivalent serum and white blood cell exposures whether a total dose of 1.5 g of azithromycin was given as a 1 day or 3 day regimen. PK-PD data have also contributed to the growing evidence base for the selection of a more rational interval, but were similar for the three regimens over the 3 day sampling period. Total serum and pulmonary exposures whether a total dose of 1.5 g of azithromycin was given as a 1 day or 3 day regimen. PK-PD data have also contributed to the growing evidence base for the selection of a more rational interval, but were similar for the three regimens over the 3 day sampling period.

The finding in this study that composite efficacy data following single-dose azithromycin in numerous animal models of infection was most closely correlated with 24 h AUC/MIC confirmed the importance of dose and not dosing interval in determining bacterial clearance and survival rates following streptococcal challenge in mice. The pharmacokinetic relationship between azithromycin and dosing interval was further explored following administration of a total dose of 100 mg/kg azithromycin, given either as a single dose or as a divided dose over 2 or 3 days. Total serum and pulmonary exposures were similar for the three regimens over the 3 day sampling interval, but C_max was dependent on the initial dose, with the single-dose regimen producing the highest C_max. These data are consistent with data previously reported by Craig, who demonstrated that a 24 h AUC/MIC ratio could be used to predict both bacteriological and clinical efficacy of azithromycin in AOM. Arguably for these azithromycin data as well as for Craig’s, the dose and C_max of azithromycin required for efficacy in murine models are considerably greater than those required in man due to the higher rate of azithromycin clearance in mice. Humanization of azithromycin pharmacokinetics in the mouse has been experimentally impractical but recently a new approach described by Forrest et al. in gerbils resulted in minimizing the large peak-to-trough plasma concentrations for a target plasma AUC and would be useful in further probing the impact of peak on efficacy of azithromycin. In addition to investigating PK-PD relationships using S. pneumoniae and S. pyogenes as challenge organisms, we also wanted to provide robust analysis of antimicrobial efficacy outcomes against a range of pathogens in different animal models. In the acute peritonitis model, bacterial strains were chosen for their ability to produce a more chronic infection amenable to the evaluation of short-course versus single-dose therapy. In the murine peritonitis model, a single-dose regimen of azithromycin was consistently superior to a 2 or 3 day regimen following challenge with S. pneumoniae, S. pyogenes, E. faecalis or H. influenzae, suggesting that dosing azithromycin less frequently is more effective than dosing more often when giving the same total dose. (The in vivo activity of azithromycin against E. faecalis was assessed to provide an example of an efficacious outcome against a non-susceptible organism despite free plasma concentrations below the pathogen’s MIC, as has also been shown with H. influenzae.) In contrast, clarithromycin, a time-dependent antibiotic, was generally less active than azithromycin irrespective of the dosing regimen and would probably benefit from a longer course of therapy. This finding probably relates to the different pharmacokinetics of the two drugs. Whereas azithromycin is rapidly absorbed into tissue and maintains high concentrations for relatively long periods, clarithromycin achieves high serum concentrations, but is rapidly eliminated. Therefore, to maximize efficacy, clarithromycin requires multiple daily dosing.

In the mouse pneumococcal pneumonia model, single-dose azithromycin again demonstrated superior efficacy to a 3 day regimen. However, in the gerbil AOM model, the ED₅₀ for the 1 day regimen was not statistically significantly different from the 2 and 3 day regimens. This may be due to the mechanism of phagocytic delivery of azithromycin. Since azithromycin concentrations at infection loci are primarily driven by phagocytic infiltration and drug deposition during inflammation, the fact that therapy was initiated prior to maximal inflammation in the gerbil model, may have favoured the 2 and 3 day regimens. Even so, all three azithromycin regimens were effective. Since the gerbil does not
metabolize clarithromycin to 14-hydroxylactithromycin and clarithromycin dosing was not optimized, clarithromycin failed in this model. For amoxicillin/clavulanate, the dosing of which was also not optimized to its PK/PD characteristics, the ED₉₀ value was non-significantly lower for a 3 day regimen.

A further set of experiments using the gerbil AOM model investigated the in vivo bacterial clearance kinetics for azithromycin 200 mg/kg delivered as a single dose or as a 2 or 3 day regimen against a penicillin-resistant H. influenzae strain. All three regimens eradicated H. influenzae from the bulla, but sterilization was more rapid using the single-dose azithromycin regimen.

These data provide support for existing clinical data demonstrating that single-dose azithromycin is at least as effective as regimens of a longer duration. In addition, the marked decrease in ED₉₀ values in murine models and the finding that H. influenzae were cleared more rapidly in the gerbil otitis media model when a single-dose regimen was administered suggests that azithromycin may perform better when administered this way. Increasing the dose of azithromycin early in the infectious process may facilitate earlier clinical cure, even in infections caused by marginally susceptible pathogens. Furthermore, such a strategy may negate the potential for selection of resistance and its associated complications.

In conclusion, this study compared the relative efficacy of single-dose versus 2 or 3 day dosing regimens of azithromycin in four different preclinical infection models using clinically relevant bacterial pathogens. These efficacy data indicate that a single-dose azithromycin regimen is better than a longer course of therapy. Additionally, the in vivo bacterial clearance kinetics of azithromycin against H. influenzae suggest that bacterial clearance is more rapid with a single-dose regimen, which could have benefits in minimizing the emergence of resistance. These preclinical data correspond to what has been observed in clinical studies and highlight the advantages of 1 day azithromycin dosing over a more prolonged course of therapy.

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References