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Comparison of Pharmacodynamics of Azithromycin and Erythromycin In Vitro and In Vivo

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In this study, we determined the efficacy of various dosing regimens for erythromycin and azithromycin against four pneumococci with different susceptibilities to penicillin in an in vitro pharmacokinetic model and in a mouse peritonitis model. The MIC was 0.03 μg/ml, and the 50% effective doses (determined after one dose) of both drugs were comparable for the four pneumococcal strains and were in the range of 1.83 to 6.22 mg/kg. Dosing experiments with mice, using regimens for azithromycin of one to eight doses/6 h, showed the one-dose regimen to give the best result; of the pharmacodynamic parameters tested (the maximum drug concentration in serum [C_{max}], the times that the drug concentration in serum remained above the MIC and above the concentration required for maximum killing, and the area under the concentration time curve), C_{max} was the best predictor of outcome. The bacterial counts in mouse blood or peritoneal fluid during the first 24 h after challenge were not correlated to survival of the mice. The serum concentration profiles obtained with mice for the different dosing regimens were simulated in the in vitro pharmacokinetic model. Here as well, the one-dose regimen of azithromycin showed the best result. However, the killing curves in vivo in mouse blood and peritoneal fluid and in the vitro pharmacokinetic model were not similar. The in vitro killing curves showed a decrease of 2 $\log_{10}$ within 2 and 3 h for azithromycin and erythromycin, respectively, whereas the in vivo killing curves showed a bacteriostatic effect for both drugs. It is concluded that the results in terms of predictive pharmacodynamic parameters are comparable for the in vitro and in vivo models and that high initial concentrations of azithromycin favor a good outcome.

Although macrolides are being used to treat moderate to severe infections, it is not well known how effective these drugs are in the treatment of infections that are accompanied by a severe sepsis syndrome (5, 9, 11, 14, 15, 24, 26). One of the problems is that the volume of distribution of these drugs is quite large, resulting in relatively low concentrations in serum (10, 18, 22). Thus, the relationship between the concentration in serum and the MIC for the infecting microorganism never attains high values and remains questionable. This is especially true for the recently clinically introduced 15-membered macrolide the azalide azithromycin, which has an even greater volume of distribution. For example, the range of azithromycin concentrations in tissue is 1 to 9 mg/kg, which is 10 to 100 times the concentration in serum (6, 7, 30). The importance of the high ratio of the concentration in serum to the MIC has not been established for azithromycin. Since the occurrence of penicillin resistance in pneumococci (16), the quest for knowledge about the efficacy of alternative drugs in the treatment of pneumococcal disease is warranted.

There are several ways to shed light on this issue by using a mouse sepsis model, the survival of mice or the bacterial counts can be determined, and by measuring the concentrations of the macrolides in the different body compartments, the relationship between drug concentration and efficacy can be determined. Although this approach has been used in several animal models, in none of these models was a severe sepsis syndrome present (1, 3, 23, 29). Another approach would be to simulate the pharmacokinetics of the macrolides in an in vitro pharmacodynamic model and determine the antimicrobial efficacy of macrolides given in several dosing regimens. By combining the results of in vitro and in vivo efficacy experiments, more detailed insight into the pharmacodynamic principles of macrolides can probably be gained. Such a combined approach would also be of value when defining breakpoints for in vitro susceptibility testing with routine laboratory tests. However, the usual method of relating MICs directly to concentrations in serum can obviously be applied to macrolide drugs only within certain limits.

The purpose of the present study was twofold. The first goal was to investigate the efficacy of one of the recently clinically introduced macrolides, azithromycin, in the treatment of a severe sepsis syndrome and to determine which pharmacokinetic and pharmacodynamic parameters are the best predictors of efficacy.

The second objective of the study was to compare data derived from an animal infection model with those determined in an in vitro pharmacodynamic model. There is, as far as we know, only one previous study comparing the results of an in vitro model with those of an animal model (2). However, in that study, efficacy, as measured by the killing effect, was comparable in both models but macrolides were not used.

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MATERIALS AND METHODS

Strains, antibiotics, and media. The strains used for the experiments were four clinical isolates of *Streptococcus pneumoniae* with different susceptibilities to penicillin. The serotypes were determined at The Streptococcus Department, Statens Serum Institut (Copenhagen, Denmark), by using anticapsular polysaccharide antibodies (19). Erythromycin (E 6376; Sigma Chemical Company, St. Louis, Mo.) and azithromycin (azithromycin dihydrate; Pfizer Pharmaceuticals,

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TABLE 1. In vivo and in vitro efficacies of azithromycin and erythromycin against four *S. pneumoniae* strains

Strain/ serotype	MIC (μg/ml)			ED_{50} (mg/kg) (95% CI)[a]		Generation time (min) in MHB in vitro
	Azithromycin	Erythromycin	Penicillin	Azithromycin	Erythromycin	
68040/6B	0.03	0.03	0.016	1.83 (0.34–4.90)	3.75 (1.27–8.28)	53
964/14	0.03	0.03	0.25	3.87 (1.49–8.10)	3.48 (0.54–13.55)	44
999/19A	0.03	0.03	0.5	6.22 (2.95–12.97)	4.15 (1.18–10.51)	31
1064/6B	0.03	0.03	0.25	4.84 (4.84–4.84)	2.15 (0.37–6.04)	31

[a] ED_{50}s were determined in mice. CI, confidence interval.

Ringasskiddy, Ireland) were used and dissolved in accordance with the manufacturers' instructions. All in vitro experiments were performed in Mueller-Hinton broth (MHB; Difco, Amsterdam, The Netherlands). Todd-Hewitt broth (Difco) was used to culture pneumococci prior to the time-kill experiments, and beef broth (Statens Serum Institut) was used as the medium for pneumococcal cultures prior to mouse experiments. All experimental samples were plated on 5% blood agar plates (Sanofi Pasteur [Maassluis, The Netherlands] and Statens Serum Institut).

MICs, generation time, and conventional time-kill curves. MICs of erythromycin and azithromycin were determined by using a standard agar dilution method (21), a macrodilution method (21), and the gradient disk diffusion method (E test; AB Biodisk, Solna, Sweden). The generation times of all strains were determined during conventional logarithmic growth in tubes of MHB. Conventional time-kill experiments were performed with erythromycin and azithromycin at concentrations of 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, and 128 times the MIC in shaking tubes containing 10 ml of MHB. All time-kill curves were determined in duplicate. For each experiment, a fresh culture was made in 30 ml of Todd-Hewitt broth inoculated with 5×10^5 CFU of a standardized pneumococcal batch stored at −80°C. After 12 h of incubation at 37°C, these cultures were diluted in prewarmed MHB and shaken for 1.5 h at 37°C, resulting in a logarithmic-phase culture of 5×10^5 CFU/ml. Samples were then diluted with prewarmed MHB containing twice the final antibiotic concentration. Samples were subsequently taken at $t = 0$, 1, 2, 4, and 6 h, and the numbers of CFU per milliliter were determined after making appropriate 10-fold dilutions in cold phosphate-buffered saline (pH 7.4). From each dilution, 0.1 ml was plated on a 5% blood agar plate and incubated for 48 h at 37°C (limit of detection, 10 CFU/ml).

Mouse peritonitis model. All animal experiments were approved by the animal ethical committee. The model was previously described in detail (17). Briefly, outbred female *ssc*:CF1 mice (age, approximately 8 weeks; weight, 30 ± 2 g) were used throughout the study. The mice were kept at five per cage and had free access to chow and water. From fresh overnight cultures on 5% blood agar plates, an inoculum was prepared immediately before inoculation by suspending colonies in sterile beef broth medium and diluting it to a suspension containing approximately 2×10^6 CFU/ml. Mucin (M-2378; Sigma Chemical Company) was used as an adjuvant for inoculation of the mice. Immediately before inoculation, the mucin solutions were diluted 1:1 with the pneumococcal suspensions, yielding a final mucin concentration of 5% (wt/vol). The final number of CFU per milliliter in the inoculum was determined by plating on 5% blood agar. Inoculation was performed by intraperitoneal injection of 0.5 ml of the inoculum. Blood samples were obtained by cutting the axillary artery after anesthetizing the mice with CO_2. After the mice were sacrificed, peritoneal washes were performed by intraperitoneal injection of 2 ml of sterile saline, and after the abdomen was massaged, the peritoneum was opened for fluid collection (8). Blood and peritoneal fluids were immediately diluted, and duplicate 20-μl samples were plated in spots on 5% blood agar plates. Mice were treated by administering subcutaneous injections in the neck region.

Determination of the ED_{50}. The 50% effective doses (ED_{50}s) were determined by administration of one-dose treatments 1 h after challenge with pneumococci. The determinations were done in two steps for each drug and strain. In the first step, 25 mice were treated in groups of 5 with five successive 10-fold higher doses of the antibiotics. The maximum dose was 100 mg/kg. In the second step, 25 mice were treated in groups of 5 with doses within the range of the ED_{50}s estimated in the first step. A group of five control mice was included in every experiment. The drugs were administered as a single injection of 0.5 ml subcutaneously. The mice were observed for 6 days, and mortality was registered.

Pharmacokinetics in mice. Pharmacokinetic studies of erythromycin and azithromycin in healthy mice were performed. For each time point, blood was collected from three mice for determination of the antibiotic concentration. After collection of the samples, the blood was centrifuged at $1,630 \times g$ for 10 min and the serum was stored at −80°C until analysis. The cup plate or the disk diffusion bioassay method (4) was used to measure the concentrations of erythromycin and azithromycin in mouse serum. *Sarcina lutea* ATCC 9341 was used for the bioassay. The lower limit of detection was 0.125 μg/ml. The variation coefficients were below 5% for all of the bioassays used. All determinations were performed in duplicate.

Dose regimens for mice. The treatments were always initiated 1 h after challenge, a time at which the bacteria were known to be in the growth phase (17). The total dose of either erythromycin or azithromycin was 4 mg/kg, given either as one dose or divided into four doses of 1 mg/kg, with a dosing interval of two serum elimination half-lives ($t_{1/2}$s) ($t = 0$ and 80 min and $t = 0$ and 100 min, respectively). These regimens were chosen because of the difference between the $t_{1/2}$s of the two drugs and the fact that we wanted to obtain comparable regimens for the drugs. In mouse survival studies, the same dose of 4 mg/kg was given as one, two, four, or eight doses of azithromycin (i.e., 4, 2, 1, and 0.5 mg/kg, respectively), with dose intervals of 4, 2, and 1 times the $t_{1/2}$, respectively. Erythromycin was given once or as four doses with an interval of twice the $t_{1/2}$. A group of control mice was included in every experiment.

In vitro model. The in vitro model used was described previously in detail (20). Briefly, a two-compartment model consisting of one central compartment and

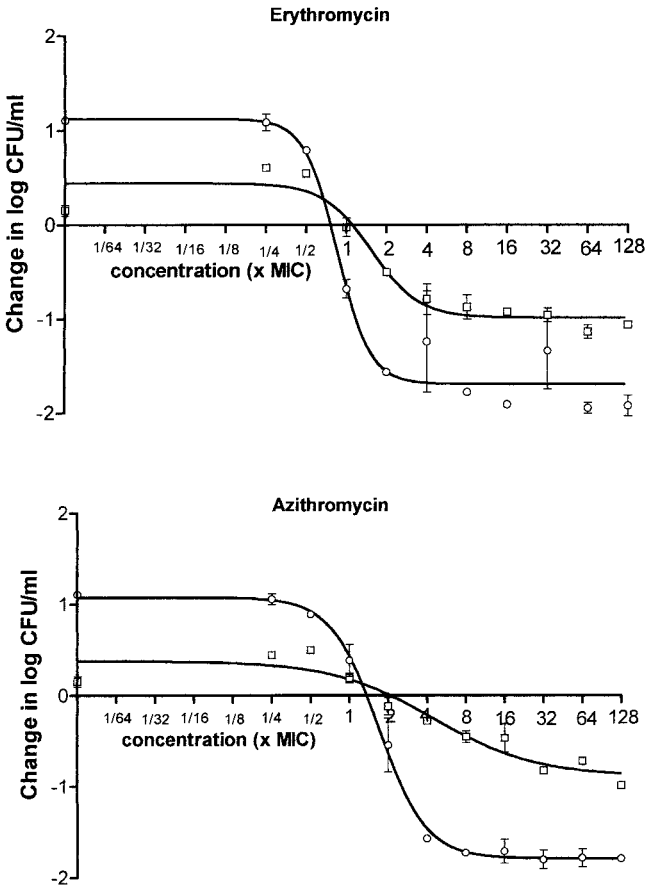

FIG. 1. Growth and killing of *S. pneumoniae* 1064 exposed to increasing concentrations of erythromycin (top) or azithromycin (bottom). The change in log CFU is the difference in CFU at $t = 0$ h and at 1 h (□) or 4 h (○), respectively. The symbols indicate the observed CFU, and the curves are fits obtained by using a sigmoidal dose-response equation with a variable slope. The data are means of two separate experiments.

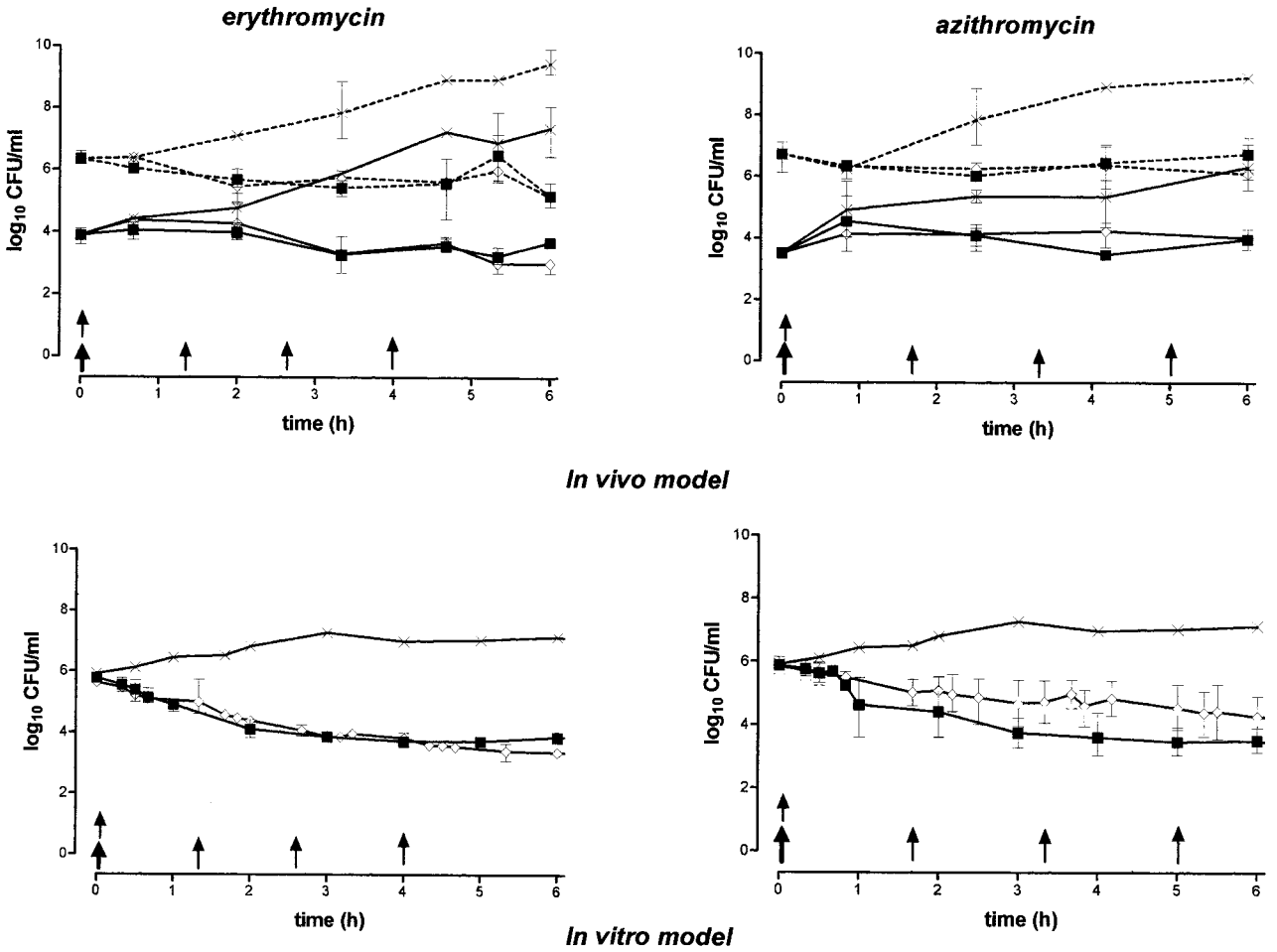

FIG. 2. Killing of *S. pneumoniae* 1064 in mice and in the in vitro model. Control data on the growth of the strain in vitro (×) and in the blood (—×—) and peritoneums (---×---) of mice are shown in all of the graphs. The symbols correspond to exposure to erythromycin in one dose (■) or in four divided doses (◇). For the in vivo experiments, curves indicated by solid and broken lines indicate the numbers of CFU in blood and the peritoneum, respectively.

four peripheral compartments consisting of disposable dialyzer units (ST23; Baxter, Utrecht, The Netherlands) was used to expose the bacteria in the peripheral compartments to declining antibiotic concentrations that vary according to mouse pharmacokinetics. One hundred fifty milliliters of a logarithmic-phase culture containing 5×10^5 CFU/ml (prepared freshly as described above) was injected into the peripheral compartments of the in vitro model. Samples were taken at the intervals indicated in Results for determination of CFU counts and antibiotic concentrations. The peak concentrations (C_{max}), the time to the C_{max} (T_{max}), and the $t_{1/2}$ of the antibiotics in the model were adjusted to those found in the mouse model. Antibiotic treatment was started at $t = 0$ h with an infusor (Braun AG, Melsungen, Germany).

Dose regimens in vitro. The erythromycin and azithromycin regimens used for the mice were simulated in the in vitro model (see above). Samples were taken every hour starting at $t = 0$ h. At 10 and 20 min after the C_{max} was reached, additional samples were taken for antibiotic concentration determination. Antibiotic concentrations were determined by using the cub agar diffusion method as described above. These concentrations were used to check the C_{max} and $t_{1/2}$. All regimens were performed in quadruplicate.

Analysis and statistical methods. The logit transformation was used to calculate the ED_{50} (27). The $t_{1/2}$s of erythromycin and azithromycin in mice and in the vitro model were estimated from the expression $-\log 2/\beta$, where β is the slope of the serum elimination regression line (time versus the log of the concentration in serum). From the conventional time-kill curves, the minimum concentrations of the drugs given the maximal achievable killing of the pneumococcus were defined. The C_{max}, the T_{max}, and the times that the drug concentration in serum remained above the MIC ($T_{>MIC}$) and above the concentration required for maximum killing ($T_{>max\text{-}kill}$) were estimated from the serum elimination regression line. A simulation of the antibiotic concentration profile during all experiments was done by using the formula of an open-compartment model after extravascular administration (25). The area under the concentration-time curve (AUC), $T_{>MIC}$, and $T_{>max\text{-}kill}$ (i.e., time above a concentration equivalent to four times the MIC) were calculated by using these simulated curves.

The Hill equation with a variable slope was used to describe the dose-response curves of the conventional time-kill experiments. Statistical analysis of the bacterial killing curves (i.e., the difference between $\log_{10}$ CFU per milliliter at $t = 0$ h and $t = 6$ h), both for the in vitro model and for the conventional killing curves, was done by two-way analyses of variance and Tukey's test for multiple comparisons of significance (13).

The method of Kaplan-Meier (12) was used for evaluation of the survival data with product limit survival estimates. The log rank test was used to determine significant trends in the curves (12).

To determine which pharmacokinetic parameters are predictive of efficacy, multivariance analyses were performed by using forward and backward elimination procedures (27). The following parameters were included in the model: C_{max}, T_{max}, $T_{max\text{-}kill}$, and AUC. A P value of ≤ 0.05 (two tailed) was considered significant.

RESULTS

MICs and ED_{50}s. The capsular serotypes and in vitro generation times of the four clinical isolates of *S. pneumoniae* tested are given in Table 1, as are the MICs and the ED_{50}s of azithromycin and erythromycin. The MICs were identical for all four strains and with all three of the methods used (data not shown), and the ED_{50}s for mice were also highly comparable.

Conventional time-kill experiments. Results of time-kill experiments with strain 1064 exposed to azithromycin and

TABLE 2. Survival of mice challenged with *S. pneumoniae* 1064 and pneumococcal killing effects of erythromycin and azithromycin in vivo and in vitro

No. of doses[a]	In vivo data		In vitro change in log CFU at 6 h (95% CI)	Pharmacokinetic parameters[b]				
	No. of mice dead/total (%) within 6 days	Change in log CFU at 6 h in blood/peritoneal fluid (95% CI)[c]		C_{max} (mg/liter) ($\times$ MIC)	$T_{>MIC}$ (h)	$T_{>max\text{-}kill}$ (4 $\times$ MIC) (h)	AUC_{0-6} (mg/liter · h)	$AUC_{0-\infty}$ (mg/liter · h)
Azithromycin								
1	37/85 (44)	0.5 (0.4 to −1.3)/0.02 (−1.3 to 1.3)	−2.4 (2.8–2.1)	0.8 (26)	4.2	2.5	1.2	1.2
2	29/54 (54)	ND[d]	−1.6 (2.0–1.3)	0.4 (13)	6.9	3.6	1.2	1.2
4	30/55 (55)	0.5 (0.5 to 0.5)/−0.6 (−2.8 to −1.6)	−1.6 (2.3–0.9)	0.2 (6.5)	7.5	3.2	0.9	1.2
8	41/55 (75)	ND	−1.6 (1.7–1.5)	0.1 (3.3)	7.5	0	0.6	1.2
Erythromycin								
1	24/30 (80)	−0.2 (−0.5 to 0.1)/−1.2 (−4.9 to −0.8)	−1.9 (2.2–1.9)	0.8 (26)	3.2	2.0	1.0	1.0
4	24/30 (80)	−0.9 (−1.6 to −0.2)/−1.2 (−2.2 to −0.2)	−2.3 (2.5–2.1)	0.2 (6.5)	6.4	3.6	1.0	1.0

[a] The total amount of each drug given in each case was 4 mg/kg.
[b] Calculated from simulated curves.
[c] CI, confidence interval.
[d] ND, not done.

erythromycin in vitro are given in Fig. 1. The change in $\log_{10}$ CFU per milliliter was plotted against the concentrations as multiples of the MIC and then fitted to the Hill equation. The curves fitted to the counts obtained after 4 h of exposure show that maximum killing was reached at four times the MIC. In contrast, if 1-h exposure values are used, maximum killing by azithromycin was reached only at 128 times the MIC, indicating that there is concentration-dependent killing if strains are only briefly exposed to the drug. This phenomenon was not observed with strain 964; otherwise, the results were similar to those obtained with strain 1064 (data not shown).

Pharmacokinetics in mice and in the in vitro model. The T_{max}, C_{max}, and $t_{1/2}$ (mean ± standard deviation) determined with mice for azithromycin and erythromycin were 10 to 20 min, 0.8 to 1.0 μg/ml, and 43 ± 8 and 51 ± 10 min, respectively. On the basis of these observations, the pharmacokinetic profiles of the free fractions of these drugs were simulated in the in vitro model. The C_{max} in the model was adjusted to 0.8 mg/liter for both drugs, taking into account approximately 20% protein binding for erythromycin and <8% for azithromycin (28). There were no significant differences between the observed relevant pharmacokinetic parameters in vivo and in vitro. The actual drug concentrations determined during the experiments fitted well in the simulated drug-time profile for all regimens (data not shown).

Efficacy studies. (i) CFU counts. In mice, the efficacy of a 4-mg/kg dose of azithromycin or erythromycin, administered either in one dose or in divided doses, was determined by CFU counts in blood and in peritoneal fluid taken at intervals of up to 6 h. The number of CFU in blood generally followed the same time course as that found in peritoneal fluid (Fig. 2). Erythromycin had only a slight bactericidal effect both in blood and in the peritoneum, as was true for azithromycin as well. At $t = 6$ h, no significant difference between the two dose regimens of either macrolide could be demonstrated.

When the same dosing regimens were used in the pharmacokinetic model, there was no apparent difference between the one-dose regimen of erythromycin and the same dose divided into four doses. In contrast, for azithromycin the one-dose regimen was significantly more efficacious than the four-dose regimen ($P = 0.02$). This difference became apparent after the first hour of exposure.

Comparison of the data for erythromycin and azithromycin showed better in vitro killing by azithromycin given as one dose than by one dose of erythromycin ($P = 0.01$). However, no significant difference in efficacy was observed between the other azithromycin and erythromycin dosing regimens. Experiments with strain 964 showed similar results (data not shown).

(ii) Pharmacodynamics in mice versus in vitro. The survival rates of mice observed for 6 days after treatment with the different regimens showed that there was a difference in efficacy between the different dosing regimens of azithromycin (Table 2). Kaplan-Meier survival analysis showed that there was a trend for increased survival when azithromycin was administered less frequently ($P = 0.001$). In contrast, survival of mice was not different for the erythromycin regimens ($P = 0.83$).

The correlation between survival of mice and bacterial counts was studied during the dosing experiments. We did not find any correlation between survival of mice and bacterial counts either in blood or in peritoneal fluid. In one of the experiments, the counts were measured during the treatment period (Table 2). In another of these dosing experiments, the counts were determined in five mice from each treatment group 24 h after challenge. The results, given as $\log_{10}$ counts in blood and peritoneal fluid, as medians and ranges, and the 6-day survival of mice treated equally, are shown in Table 3. There was no significant correlation between bacterial killing at $t = 24$ h and survival of mice.

To determine which of the pharmacodynamic parameters (AUC, $T_{>MIC}$, C_{max}, or $T_{>max\text{-}kill}$) was most predictive of the outcomes of the different azithromycin regimens in vivo and in vitro, a multivariance analysis was performed, despite the few data sets. Both for survival and for killing in the in vitro model, the C_{max} appeared to be the most significant predictive parameter ([$P = 0.001$] and $R^2 = 0.49$ [$P = 0.003$], respectively). In the in vitro model, the coefficients of determination for the other pharmacodynamic parameters had R^2 values of 0.10 ($P = 0.24$), 0.35 ($P = 0.015$), and 0.40 ($P = 0.009$) for $T_{>max\text{-}kill}$, AUC, and $T_{>MIC}$, respectively. For the survival experiment, the P values of the survival analysis were 0.006 and 0.072 for $T_{>max\text{-}kill}$ and $T_{>MIC}$, respectively. For the AUC, no calculations were possible in vivo since we used only one dose. For erythromycin, such an analysis was not possible due to the smaller number of dosing regimens tested.

TABLE 3. Bacterial counts at $t = 24$ h and survival of mice during one experiment using different azithromycin dosing regimens

No. of doses[a]	Log median blood CFU count (range)	Log median peritoneal CFU count (range)	No. of mice dead/total (%) within 6 days
0 (control)	4.23 (1.70–7.43)	6.68 (5.38–11.11)	10/10 (37.5)
1	4.10 (3.23–6.89)	7.26 (6.99–8.95)	10/25 (40)
2	1.70 (1.70–2.35)	4.38 (1.11–5.75)	9/24 (37.5)
4	3.72 (1.88–4.94)	6.58 (2.77–7.70)	14/25 (56)
8	3.80 (3.04–5.18)	9.88 (5.78–9.95)	16/25 (64)

[a] The total amount of azithromycin administered in each case was 4 mg/kg.

DISCUSSION

In this study, we evaluated the pharmacodynamic parameters of efficacy for macrolide antibiotics (azithromycin and erythromycin) in a mouse model of a severe sepsis syndrome due to bacteremial pneumococcal infection and compared these with the same parameters in an in vitro pharmacokinetic model. In both models, the azithromycin C_{max} was most predictive of success, indicating that large doses given infrequently are better than the same amount of the drug given in multiple doses with shorter dosing intervals.

The results of the conventional in vitro time-kill experiments indicate that the maximum bactericidal effect of azithromycin is reached at four times the MIC. However, there appears to be a greater concentration-dependent effect during the first hour of exposure to azithromycin. This effect disappears after 1 to 4 h. One of the explanations could be that there is some kind of concentration-dependent uptake of azithromycin in the cell. If this is the case, it could be argued that the first dose of azithromycin should be high. On the other hand, the maximum effect after 1 h is only 1 $\log_{10}$ decrease whereas a 2 $\log_{10}$ decrease is achieved after 4 h; thus, the net initial effect of a high first dose would probably be marginal.

The killing experiments performed with the in vitro pharmacokinetic model showed a significantly better result when azithromycin was given as one dose than when it was given in a multiple-dose regimen. This benefit of one dose became apparent during the first hour of exposure (Fig. 2). We calculated that the C_{max} reached during the one-dose experiment corresponds to 16 to 32 times the MIC. Beyond 1 h, the kinetics of killing more or less paralleled that of the other dosing regimens, which contrasted with the progressive killing observed in the conventional time-kill experiments. This difference can be explained by the decreasing concentrations of azithromycin in the pharmacodynamic model, as opposed to the static concentrations in the conventional killing experiments.

In vivo, the initial effects of azithromycin on the CFU counts in blood and the peritoneum were quite similar, irrespective of the dosing regimen. There was no obvious relationship with in vivo efficacy and the data obtained with the in vitro model. An explanation could be that the pneumococcal growth rates are significantly different in the two systems.

Comparison of bacterial killing in vitro and in mice showed significant differences both during the 6 h of treatment and 24 h after challenge. The same factors as just mentioned to explain the in vivo results may be responsible for this. One way to obtain more comparable results would be to reproduce the exact in vivo growth rate of pneumococci in the in vitro model, for instance, by adjusting the composition of the medium. Another possibility is to compare killing curves obtained with the in vitro and in vivo models after correcting for differences in the rate of growth. In this case, the observed difference between in vitro and in vivo killing by azithromycin disappears (results not shown). Although this approach seems attractive, the results may become highly dependent on differences in growth rate and may poorly reflect the antimicrobial activity of the agent itself. We conclude that initial bacterial killing rates obtained with the two models are not directly comparable.

The results of the mouse survival experiments showed that azithromycin administered as one dose significantly increased the survival rate compared with all of the other regimens. Furthermore, trend analysis showed that survival was inversely related to the number of divided doses given. This indicates that the C_{max} may be an important pharmacodynamic parameter for prediction of clinical efficacy. Other pharmacodynamic parameters did not show such a consistent relationship with survival. These results are in agreement with those obtained from the pharmacodynamic model, as regression analysis of the in vitro results likewise showed the C_{max} to be the single significant parameter that explains the efficacy of azithromycin.

Thus, although the initial (6-h) killing rates obtained with the two models are not directly comparable, the final conclusion with regard to the pharmacodynamics of azithromycin are the same. This was also shown for another in vitro and in vivo model comparison using other antibiotics (2).

The data for the two erythromycin regimens tested (Table 2) showed no significant difference in either the survival data or the killing data from the in vitro model.

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