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Azithromycin Pharmacokinetics and Intracellular Concentrations in *Legionella pneumophila*-Infected and Uninfected Guinea Pigs and Their Alveolar Macrophages

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Azithromycin pharmacokinetics in *Legionella pneumophila*-infected and uninfected guinea pigs were assessed by measuring the drug concentration in whole lungs or the drug content in bronchoalveolar lavage (BAL) fluid in separate experiments. Azithromycin concentrations were measured by using a bioassay. The mean azithromycin content in the BAL fluid of infected guinea pigs was higher than that in controls at 10 h (0.87 versus 0.39 μg; $P = 0.05$), 24 h (1.10 versus 0.37 μg; $P = 0.003$), and 48 h (1.21 versus 0.28 μg; $P = 0.05$) after a single intraperitoneal injection of drug (15 mg/kg). The mean peak lung azithromycin concentration was higher in control animals than in infected animals (15.8 versus 13.4 μg/ml). The mean lung azithromycin concentration in infected animals was significantly higher than that in controls 48 h after dosing (12.7 versus 10.4 μg/g; $P = 0.04$). There were no significant differences between infected and uninfected animals in serum azithromycin levels. Complementary experiments assessed intracellular/extracellular concentration ratios of azithromycin and erythromycin in *L. pneumophila*-infected and control guinea pig alveolar macrophages. Azithromycin was highly concentrated in alveolar macrophages, and the intracellular/extracellular concentration ratios for infected cells were significantly higher ($P < 0.0001$) than those observed in controls after 4 h (127 versus 119), 24 h (481 versus 361), and 48 h (582 versus 520) of incubation. Erythromycin was also preferentially concentrated in infected cells ($P < 0.0001$). AZ intracellular concentrations were at least fivefold higher than those measured for erythromycin, and this differential increased with incubation time. Thus, azithromycin recovery from BAL fluid, and from guinea pig lungs at the 48-h time point, was higher in the presence of experimental Legionnaires' disease. This likely results from recruitment of phagocytes, including macrophages, that have an enhanced capacity to highly concentrate the drug.

Azithromycin is a 15-membered-ring azalide synthesized from erythromycin (4, 23). This antimicrobial agent differs from its parent compound in that it has fewer gastrointestinal side effects, a longer half-life, and improved activity against gram-negative bacteria, including *Legionella pneumophila* (16, 21, 24, 25, 31). While tissue (17) and phagocyte (19, 30) azithromycin concentrations are much higher than the corresponding erythromycin concentrations, the serum azithromycin levels are low but sustained (7, 16, 22). This is felt to be due to the dibasic nature of azithromycin and its concentration within cells on the basis of pH partition (9, 20). Because of its high intracellular concentrations, azithromycin is a promising alternative to erythromycin for treating Legionnaires' disease, which is caused by an intracellular bacterium. In support of this, Fitzgeorge and colleagues demonstrated that two oral doses of azithromycin protected guinea pigs against lethal challenge with *L. pneumophila*, whereas erythromycin treatment was much less effective (15). On the basis of the foregoing information and the knowledge that phagocytes are recruited to the alveoli of guinea pigs with experimental Legionnaires' disease (8), we hypothesized that lung azithromycin concentrations in infected guinea pigs would exceed those in uninfected control animals. To test this hypothesis, we conducted separate studies to measure the azithromycin concentration in lungs or the azithromycin content in bronchoalveolar lavage (BAL) fluid in *L. pneumophila*-infected and control guinea pigs. Complementary experiments were performed to assess the abilities of *L. pneumophila*-infected and uninfected macrophages to concentrate azithromycin. Our findings demonstrate that *L. pneumophila*-infected guinea pigs contain more azithromycin in their BAL fluid than do controls and that this difference parallels the augmented intracellular concentration of azithromycin in infected as opposed to uninfected macrophages.

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MATERIALS AND METHODS

Bacterial inoculum. *L. pneumophila* serogroup 1 (strain F889) was used for all studies. It was grown on buffered charcoal yeast extract broth (for animal studies) or agar (for macrophage studies) supplemented with α-ketoglutarate, as previously described (10). The actual infecting inocula used for the animal and macrophage studies were determined by viable plate counting.

Animal infection. Male Hartley strain guinea pigs were quarantined and observed prior to experimental infection, as previously described (12). Animals were challenged with either 4.7×10^6 CFU (whole-lung study) or 1.1×10^7 CFU (BAL study) of *L. pneumophila* delivered by the intratracheal route, using methods previously described (12). The different challenge doses were not intentional but rather were because of experimental error; both challenge doses would be expected to result in >95% mortality within 5 days postinfection.

Pharmacokinetics. (i) Whole-lung study. Guinea pigs were randomly assigned to be experimentally infected (n = 15) or to serve as controls (n = 12). Twenty-four hours following challenge, all guinea pigs were weighed and were dosed with azithromycin (Pfizer Pharmaceuticals, New York, N.Y.) on the basis of their individual weights. Each animal received a single intraperitoneal injection of azithromycin (15 mg/kg) dissolved in sterile phosphate buffer (pH 6.5, 0.1 M). At timed intervals following drug administration (0.5, 1, 4, 10, 24, and 48 h), two animals from each group were killed by a lethal injection of pentobarbital, and lung and heart blood samples were collected and processed as described previously (14), with the following modifications. Lung specimens were ground in sterile phosphate buffer (pH 8.0, 0.1 M), and the homogenates were centrifuged at 14,500 × g for 5 min. The supernatants were frozen at −70°C until drug concentrations were measured.

(ii) BAL study. Guinea pigs were randomized to the infection group (n = 11) or the control group (n = 9) and were dosed with azithromycin as in the whole-lung experiment. At 10, 24, and 48 h after drug administration, three or four animals from each group were killed as described above, BAL was performed, and heart blood was obtained. BAL was performed under aseptic conditions by lavaging the lung with 20 ml of sterile phosphate-buffered saline (pH 7.4, 0.1 M) in 5-ml aliquots as described previously (11). All the recovered BAL fluid was pooled and then centrifuged in a 50-ml sterile conical tube at 300 × g for 10 min. The supernatant was discarded, and the pellet, containing alveolar macrophages, was resuspended in sterile phosphate buffer (pH 8.0, 0.10 M). The volume of the suspension was measured, and the suspension was then pulse sonicated under aseptic conditions for 30 s at an energy setting shown previously to completely disrupt macrophages (unpublished data). Suspensions were checked microscopically to ensure that cells had been lysed. Samples were frozen at −70°C until azithromycin concentrations were measured. Because drug concentrations were determined by using a bioassay, the lysate was checked for the presence of non-*L. pneumophila* bacteria by plating a loopful (1 μl) of the lysate on 5% sheep's blood Trypticase soy agar; contaminated specimens were membrane filtered (0.22-μm nylon filter; MSI, Westboro, Mass.) before being assayed for drug.

Drug bioassay. Azithromycin concentrations in serum, lung homogenate supernatant, and BAL fluid were measured by using a modified agar diffusion bioassay (17, 26). Standard curves were derived by using azithromycin concentrations ranging from 0.04 to 40 μg/ml. Standards were tested in quintuplicate, and animal specimens were tested in quadruplicate. A mean zone diameter was used to calculate each drug concentration. Separate standard curves were generated for each fluid tested, using that fluid as a diluent. Specimens requiring filtration were analyzed by using a regression curve based on the data from the filtered azithromycin standards. All samples of the same type were assayed, and the appropriate standard curve was generated, on the same day. The standard curves were linear from 0.08 to 40 μg/ml. All standard curves had correlation coefficients of 0.98 except for the one curve used to determine azithromycin concentrations in BAL fluid, which had a correlation coefficient of 0.96. The within-run coefficients of variation for the two lowest (0.08 and 0.156 μg/ml) and the two highest (1.25 and 2.50 μg/ml) standard concentrations used were, respectively, 15.0, 4.3, 5.0, and 3.4% for the curve used to determine azithromycin concentrations in BAL fluid. For the curve used to determine lung azithromycin concentrations, the coefficients of variation were 4.8% (0.6 μg/ml), 3.2% (1.25 μg/ml), 1.9% (20 μg/ml), and 0.9% (40 μg/ml). Coefficients of variation for the two lowest (0.08 and 0.156 μg/ml) and the two highest (1.25 and 2.5 μg/ml) standard concentrations used for the curve that was used to determine concentrations in serum were in the range of 3.2 to 5.2%. The lower limit of azithromycin detection was 0.08 μg/ml for all standards. In a pilot study, the efficiency of azithromycin recovery from seeded lungs was 96% for the range of lung drug concentrations measured in experimental animals. A mid-range dilution (1.25 μg/ml) of azithromycin was placed in the same location on all plates, and the zone size created by this concentration was used to normalize each plate with its appropriate standard curve. Azithromycin concentrations were calculated from corrected zone diameters by using the standard curve appropriate for each sample. Unless otherwise noted, drug concentrations represent mean values for each time point and are expressed as micrograms per gram for lung and as micrograms per milliliter for serum. Because resuspension volumes were variable for BAL cellular pellets, azithromycin concentrations were converted to total micrograms, representing the drug content in the entire BAL specimen.

Macrophage studies. Alveolar macrophages were harvested from male Hartley strain guinea pigs by BAL and were infected with *L. pneumophila* as previously described (11, 13). Methods for macrophage purification and infection were identical to those previously described, except that fetal calf serum was obtained from Hyclone (Logan, Utah) and medium 199 (J & R Scientific, Woodland, Calif.) replaced RPMI 1640 medium (11). Purified macrophages (1.5×10^6 cells per well) were suspended in medium 199 with 20% fetal calf serum (1 ml) in each well of a 24-well tissue culture tray (Flow Laboratories, McLean, Va.). Macrophages were incubated with *L. pneumophila* (8.5×10^4 CFU per well) overnight at 35°C in 5% CO_2. Infected and control macrophages were next incubated in the presence of ^{14}C-labelled azithromycin (0.06 μCi/μg; Pfizer Central Research Division, Groton, Conn.) or erythromycin (0.08 μCi/μg; DuPont NEN Research Products, Boston, Mass.), each at a concentration of 10 μg/ml. Experiments were done in triplicate, and both intracellular and extracellular concentrations of ^{14}C-labelled azithromycin and erythromycin were measured at 4, 24, and 48 h after drug exposure, according to the methods of Gladue and colleagues (19). For each time point and drug, the culture medium from all three wells was removed and pooled with four subsequent warm deionized water washes of the wells. This represents extracellular drug. The macrophages were then lysed with 0.05% Triton X-100 (Sigma, St. Louis, Mo.) in deionized water, and the lysate and four subsequent washes, representing intracellular drug, were pooled. The intracellular and extracellular pools were each combined with scintillation fluid (Ecolite Plus; ICN Flow, Costa Mesa, Calif.), and radioactivity was counted with a liquid scintillation system (LS6800; Beckman, Irvine, Calif.). Measurements were adjusted for quenching and monitored for random scatter irradiation. The mean intracellular volume of alveolar macrophages was determined by using impedence flow cytometry (Coulter Counter, S-Plus, STKR; Coulter Electronics, Hialeah, Fla.) and was 2.0×10^{-13} liter per cell. The intracellular/extracellular drug concentration ratios (I/E ratios) were then calculated.

Statistical analysis. Mean values for in vivo data were compared by using an unpaired two-tailed Student's t test, unless otherwise stated. The Mann-Whitney two-sample test or Welch's approximate t test (both two tailed) were used to compare mean values that had dissimilar standard deviations. t tests, linear regressions, data transformations, curve fittings, estimations of elimination half-lives, and calculations of areas under curves were performed by using GraphPad Instat soft-

TABLE 1. Average concentrations of azithromycin in lungs and sera of guinea pigs with *L. pneumophila* pneumonia or no infection after a single intraperitoneal administration of 15 mg/kg

Time after drug administration (h)	Concn[a] in:			
	Lungs (μg/g)		Sera (μg/ml)	
	Infected animals	Control animals	Infected animals	Control animals
0.5	9.5 (6.9–12.1)	10.4 (9.2–11.6)	0.94 (0.88–1.00)	0.95 (0.95)
1	11.6 (11.1–12.1)[b]	15.2 (14.8–15.5)	0.58 (0.45–0.71)	0.55 (0.55)
4	9.4 (8.6–10.2)[b]	13.7 (13.6–13.8)	0.46 (0.40–0.51)	0.34 (0.29–0.34)
10	13.4[c]	15.8 (14.6–16.9)	0.22[c]	0.16 (0.16)
24	11.4 (10.4–12.4)	11.3 (11.0–11.6)	0.21 (0.17–0.25)	0.15 (0.13–0.17)
48	12.7 (12.4–13.0)[b,d]	10.4 (10.1–10.8)	0.11 (0.09–0.12)[d]	0.09 (0.09)

[a] Values in parentheses represent the range. Unless noted, two animals were used for each condition.
[b] $P < 0.05$ for comparison with same-time control lung drug concentration.
[c] One animal.
[d] Three animals were used for this condition.

ware (version 2.0, 1993; GraphPad, San Diego, Calif.) or GraphPad InPlot software (version 4.0, 1993). Intracellular drug concentration measurements were assumed to follow a Poisson distribution, as they were measured by using liquid scintillation counting of radioactivity. A z test was used to compare mean intracellular counts under different conditions (27).

RESULTS

Azithromycin concentrations in lungs. Lung and serum azithromycin concentrations are shown in Table 1. The mean concentrations in lungs in control guinea pigs were significantly higher at 10 h (15.8 μg/ml) than at 48 h (10.4 μg/ml) (P = 0.046). The mean lung azithromycin concentrations in infected guinea pigs paralleled those of control animals during the first 10 h but were significantly lower than those of controls 1 h (P = 0.03) and 4 h (P = 0.03) after drug administration. By 48 h, however, the mean lung azithromycin concentration in infected animals exceeded that in control animals (P = 0.043). There were no significant differences in serum azithromycin concentrations for control or infected animals at any time point assessed (Table 1). In infected animals, lung azithromycin concentrations increased from 24 to 48 h after drug administration. During the same time period, levels of azithromycin in serum declined. The half-life of elimination of azithromycin in the lungs could not be accurately determined because the values were relatively stable over the observation period. Infected animals eliminated azithromycin from their sera somewhat more slowly than did uninfected animals, with respective half-lives of elimination being 4.7 and 3.0 h. Areas under the curves for concentrations in serum were 10.0 and 7.6 μg · h/ml for infected and uninfected animals, respectively.

Azithromycin content in BAL fluid. BAL fluid azithromycin contents are shown in Fig. 1. The azithromycin content in BAL fluid of infected animals was higher than that measured in control animals at 10 h (P = 0.05), 24 h (P = 0.003), and 48 h (P = 0.05, by Welch's approximate t test) after drug administration. The BAL fluid azithromycin content at 48 h after drug administration was more variable than were other measurements (P = 0.02 to 0.07, depending on comparison [F test]); individual values for the four samples assayed at this time point were 0.57, 1.02, 1.23, and 2.01 μg, none of which had associated aberrant serum azithromycin concentrations. The mean BAL fluid drug content in infected guinea pigs progressively increased during the study, whereas in the control group the BAL fluid azithromycin content progressively declined. Serum azithromycin concentrations were not significantly different in infected and control animals and were similar to those obtained for the whole-lung study; these values decreased during the study period for both groups.

Uptake of azithromycin and erythromycin by alveolar macrophages. Azithromycin was highly concentrated in explanted guinea pig alveolar macrophages (Fig. 2). Azithromycin accumulation, as reflected by I/E ratios, was significantly higher in infected macrophages than in uninfected controls at 4, 24, and 48 h after incubation ($P < 0.00001$) and ranged from 7 to 33% greater in infected macrophages than in controls. The intracellular concentration of azithromycin was consistently higher than that observed for erythromycin. In control macrophages at 4 h, intracellular azithromycin levels were fivefold higher than those observed for erythromycin. This differential increased to 36-fold by 48 h of incubation. In contrast to azithromycin, erythromycin did not accumulate in infected macrophages after 24 h or in control macrophages after 4 h. Erythromycin, like azithromycin, was much more highly con-

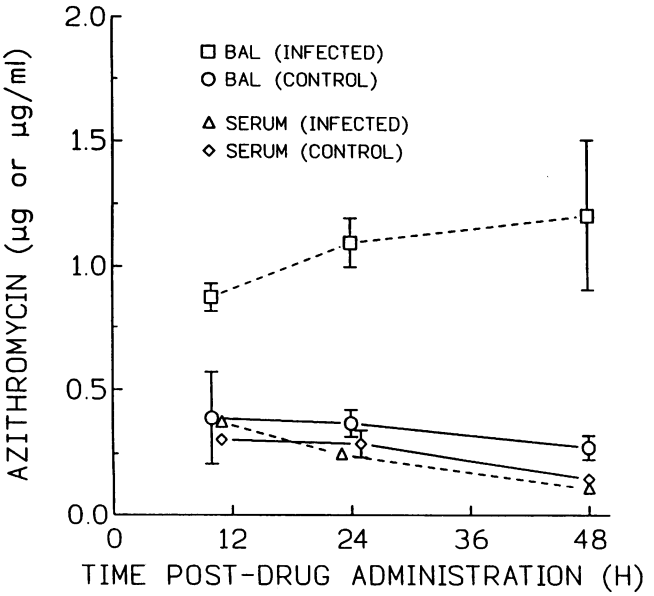

FIG. 1. Mean azithromycin contents (micrograms) in BAL fluid and mean azithromycin concentrations (micrograms per milliliter) in serum in guinea pigs with *L. pneumophila* pneumonia or no infection after a single intraperitoneal injection of 15 mg/kg. Error bars represent standard errors of the mean.

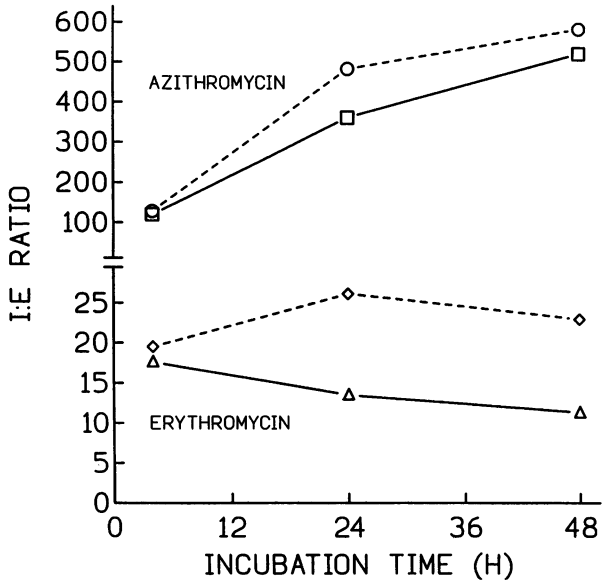

FIG. 2. I/E ratios for azithromycin and erythromycin in infected (dashed lines) or uninfected (solid lines) guinea pig alveolar macrophages following incubation in the presence of 10 μg of either antimicrobial agent per ml. The 95% confidence intervals are smaller than the symbol sizes.

centrated in infected than in uninfected macrophages at all time points tested (range, 11 to 200% greater; $P < 0.00001$).

DISCUSSION

The data that we have presented demonstrate that azithromycin is concentrated extensively in alveolar macrophages as well as guinea pig lungs and BAL fluid at a time when levels in serum are negligible. Furthermore, this extensive concentration of azithromycin is augmented by prior infection with *L. pneumophila*.

Our findings showing high concentrations of azithromycin in tissue are consistent with those of previous investigations with laboratory animals (1, 17, 18, 29). Girard and colleagues measured tissue azithromycin levels in outbred CD rats and found that following oral dosing (50 mg/kg), lung drug levels at 24 and 48 h closely approximated those that we found in this study (17). Studies conducted with uninfected mice showed peak levels of azithromycin in lungs similar to those that we found; however, these levels were attained much earlier and declined to 10% of peak concentrations by 48 h after dosing (29). This disparity may be explained by interspecies differences in drug processing or macrophage uptake of azithromycin as well as by differences in azithromycin doses and routes of administration.

Peak lung azithromycin levels in infected guinea pigs were detected at 10 h after dosing and were lower than in controls. This phenomenon has been observed by previous investigators (1, 29) and may result from enhanced systemic uptake of azithromycin by mononuclear cells. Bermudez and colleagues have shown that either gamma interferon or tumor necrosis factor alpha, both of which are released in systemic infection, increases macrophage uptake of azithromycin (3). In the present study, the mean lung azithromycin concentration in infected guinea pigs increased between 24 and 48 h and was significantly higher than that measured in controls at the latter time point ($P = 0.04$). This may be due to chemotaxis to the lung of neutrophils and macrophages, which are known to concentrate azithromycin (19). Also, azithromycin appeared to be cleared more slowly in infected animals than in controls, on the basis of the elimination kinetics of serum azithromycin, perhaps accounting in part for the higher lung azithromycin concentrations at the later time in infected animals. These observations agree in part with those from previous investigations of tissue azithromycin concentrations in experimentally infected animals (18, 29). Vallee and colleagues studied azithromycin levels in mice experimentally infected with *Haemophilus influenzae* and found that levels in lungs in infected mice were 10-fold higher than those in controls at 48 h after dosing (29). Although this increase appears to be more dramatic than the 22% increase we report, control concentrations in lungs in our study were much higher (10.4 versus 1.0 μg/g), which would tend to obscure the increment provided by phagocytic cell delivery of azithromycin into the lung. This apparent difference may result from the factors cited above for uninfected mice and also may be explained by the model of experimental infection used. We documented increasing lung azithromycin levels from 24 to 48 h after dosing in infected guinea pigs, whereas Vallee and colleagues found that lung azithromycin concentrations declined over the same time period.

The data from the BAL study show that the azithromycin content in the alveolar spaces of infected guinea pigs is greater than that in controls. Interestingly, the higher BAL fluid azithromycin values in infected as opposed to control animals at 10 and 24 h after drug administration were not associated with higher total lung drug concentrations. Vallee and colleagues found that both BAL fluid and lung azithromycin concentrations were consistently higher in mice infected with *H. influenzae* than in uninfected animals (29). They also noted that the lung azithromycin concentration paralleled the BAL cell-associated drug content. A possible explanation for our conflicting results may involve the animal models used. Vallee and colleagues employed a sublethal inoculum to induce nonparenchymatous lower respiratory tract disease, whereas we used a lethal dose that elicits exuberant pneumonia (12). The latter method causes consolidation, which likely increases lung water. The net effect may lower the concentration, but not the absolute amount, of an antimicrobial agent in the lung. Some investigators have argued that the most relevant lung tissue drug concentrations are those measured in cellular fluid (2). Therefore, measuring azithromycin in BAL fluid may be preferable to measuring the concentration of the drug in whole lungs because the former method samples alveolar macrophages and is less subject to local changes in lung tissue. Our BAL fluid measurements should accurately reflect the amount of drug present at the time of sampling, because azithromycin effluxes very slowly from macrophages in vitro, with an elimination half-life of 4 to 6 h (2, 19).

The observation that the BAL fluid azithromycin content is increased in infected guinea pigs compared with controls likely results from the abundance of phagocytes, mostly neutrophils and macrophages, in BAL fluid and their recruitment in experimental Legionnaires' disease. Davis and colleagues documented a doubling of the number of phagocytes recovered from the BAL fluid of guinea pigs 24 h following infection with *L. pneumophila* (8). In addition to recruitment of phagocytes, other factors contributing to the higher BAL fluid azithromycin level in infected guinea pigs compared with controls are that both neutrophils and macrophages avidly concentrate azithromycin (19, 30) and that, as shown here, the drug is preferentially concentrated in infected macrophages. Since the intracellular azithromycin content in macrophages obtained

from infected animals was at most 33% greater than that in macrophages from uninfected animals, in contrast to the 300 to 400% increment of the azithromycin content in BAL fluid in infected versus control animals, the greatest contributor to the high BAL fluid azithromycin levels (and the higher concentrations in lungs at 48 h) in infected animals is most likely the influx of azithromycin-containing inflammatory cells into the alveolus.

We observed much higher I/E ratios for azithromycin (361 at 24 h) than we did for erythromycin (14 at 24 h), which agrees with previous observations for humans (30) and laboratory animals (19). We also showed that both infected and uninfected macrophages continue concentrating azithromycin between 24 and 48 h of incubation. A previous investigation demonstrated marked interspecies variation in alveolar macrophage I/E ratios for azithromycin and found the I/E ratio for murine peritoneal macrophages to be 110 after 24 h of incubation with the drug (19), threefold lower than what we measured under similar conditions. This discrepancy may be explained by the fivefold larger cell volume used by these investigators. We measured cell size by using a Coulter Counter, whereas Gladue and colleagues (19) indirectly determined cell volume by measuring the volume displaced by sedimented cells. Furthermore, our cell volume measurements agree with those reported by another group of investigators (28). Regardless of the actual measurement of cell volume, the cell volume was a constant in all our calculations, and thus the differences between control and infected macrophages remain significant. Wildfeuer and colleagues studied human alveolar macrophages and observed I/E ratios for azithromycin that were twofold higher than what we observed (30). Their results may have been skewed, as the macrophages were obtained from patients with unclear smoking histories. A previous investigation has demonstrated that uptake of macrolides by macrophages was greater in smokers than in nonsmokers (6).

A key observation from our data is that azithromycin and erythromycin uptake by macrophages was enhanced by prior infection with *L. pneumophila*. Aside from the effect of cytokines on uptake of azithromycin by macrophages (discussed above), the mechanism of greater intracellular concentration of erythromycin and azithromycin by infected macrophages is unclear. The ratio of I/E ratios between infected and uninfected macrophages was 1.9-fold greater for erythromycin than for azithromycin at 48 h after drug addition. This makes increased acid trapping of these drugs by infected cells an unlikely explanation, as such trapping would be expected to have a greater effect on azithromycin than on erythromycin (9, 20). While prior infection of macrophages with *L. pneumophila* has been shown to increase intracellular accumulation of fluoroquinolones (5), until this study, the same has not been shown for erythromycin or azithromycin. Given its broad antimicrobial spectrum and its propensity to concentrate highly within infected macrophages, azithromycin has considerable potential for treating intracellular infections such as Legionnaires' disease, in addition to its known activity for the treatment of some extracellular infections.

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REFERENCES

1. **Azoulay-Dupuis, E., E. Vallee, J. P. Bedos, M. Muffat-Joly, and J. J. Pocidalo.** 1991. Prophylactic and theraputic activities of azithromycin in a mouse model of pneumococcal pneumonia. Antimicrob. Agents Chemother. **35:**1024–1028.
2. **Baldwin, D. R., D. Honeybourne, and R. Wise.** 1992. Pulmonary disposition of antimicrobial agents: methodological considerations. Antimicrob. Agents Chemother. **36:**1171–1175.
3. **Bermudez, L. E., C. Inderlied, and L. S. Young.** 1991. Stimulation with cytokines enhances penetration of azithromycin into human macrophages. Antimicrob. Agents Chemother. **35:**2625–2629.
4. **Bright, G. M., A. A. Nagel, J. Bordner, K. A. Desai, J. N. Dibrino, J. Nowakowska, L. Vincent, R. M. Watrous, F. C. Sciavolino, A. R. English, J. A. Retsema, M. R. Anderson, L. A. Brennan, R. J. Borovoy, C. R. Cimochowski, J. A. Faiella, A. E. Girard, D. Girard, C. Herbert, M. Manousos, and R. Mason.** 1988. Synthesis, in vitro and in vivo activity of novel 9-deoxo-9a-aza-9a-homoerythromycin A derivatives; a new class of macrolide antibiotics, the azalides. J. Antibiot. **41:**1029–1047.
5. **Carlier, M. B., B. Scorneaux, A. Zenebergh, J. F. Desnottes, and P. M. Tulkens.** 1990. Cellular uptake, localization and activity of fluoroquinolones in uninfected and infected macrophages. J. Antimicrob. Chemother. **26**(Suppl. B):27–39.
6. **Carlier, M. B., A. Zenebergh, and P. M. Tulkens.** 1987. Cellular uptake and subcellular distribution of roxithromycin and erythromycin in phagocytic cells. J. Antimicrob. Chemother. **20**(Suppl. B):47–56.
7. **Cooper, M. A., K. Nye, J. M. Andrews, and R. Wise.** 1990. The pharmacokinetics and inflammatory fluid penetration of orally administered azithromycin. J. Antimicrob. Chemother. **26:**533–538.
8. **Davis, G. S., W. C. Winn, Jr., D. W. Gump, and H. N. Beaty.** 1983. The kinetics of early inflammatory events during experimental pneumonia due to *Legionella pneumophila* in guinea pigs. J. Infect. Dis. **148:**823–835.
9. **de Duve, C., T. de Barsy, B. Poole, A. Trouet, P. Tulkens, and F. van Hoof.** 1974. Lysosomotrophic agents. Biochem. Pharmacol. **23:**2495–2531.
10. **Edelstein, P. H.** 1985. Legionaires' disease laboratory manual, 3rd ed. National Technical Information Service, Springfield, Va.
11. **Edelstein, P. H., K. B. Beer, and E. D. DeBoynton.** 1987. Influence of growth temperature on virulence of *Legionella pneumophila*. Infect. Immun. **55:**2701–2705.
12. **Edelstein, P. H., K. Calarco, and V. K. Yasui.** 1984. Antimicrobial therapy of experimentally induced Legionnaires' disease in guinea pigs. Am. Rev. Respir. Dis. **130:**849–856.
13. **Edelstein, P. H., and M. A. C. Edelstein.** 1989. WIN 57273 is bactericidal for *Legionella pneumophila* grown in alveolar macrophages. Antimicrob. Agents Chemother. **33:**2132–2136.
14. **Edelstein, P. H., M. A. C. Edelstein, J. Weidenfeld, and M. B. Dorr.** 1990. In vitro activity of sparfloxacin (CI-978; AT-4140) for clinical *Legionella* isolates, pharmacokinetics in guinea pigs, and use to treat guinea pigs with *L. pneumophila* pneumonia. Antimicrob. Agents Chemother. **34:**2122–2127.
15. **Fitzgeorge, R. B., A. S. Featherstone, and A. Baskerville.** 1990. Efficacy of azithromycin in the treatment of guinea pigs infected with *Legionella pneumophila* by aerosol. J. Antimicrob. Chemother. **25**(Suppl. A):101–108.
16. **Foulds, G., R. M. Shepard, and R. B. Johnson.** 1990. The pharmacokinetics of azithromycin in human serum and tissues. J. Antimicrob. Chemother. **25**(Suppl. A):73–82.
17. **Girard, A. E., D. Girard, A. R. English, T. D. Gootz, C. R. Cimochowski, J. A. Faiella, S. L. Haskell, and J. A. Retsema.** 1987. Pharmacokinetic and in vivo studies with azithromycin (CP-62,993), a new macrolide with an extended half-life and excellent tissue distribution. Antimicrob. Agents Chemother. **31:**1948–1954.
18. **Girard, A. E., D. Girard, and J. A. Retsema.** 1990. Correlation of the extravascular pharmacokinetics of azithromycin with in vivo efficacy in models of localized infection. J. Antimicrob. Chemother. **25**(Suppl. A):61–71.
19. **Gladue, R. P., G. M. Bright, R. E. Isaacson, and M. F. Newborg.** 1989. In vitro and in vivo uptake of azithromycin (CP-62,993) by phagocytic cells: possible mechanism of delivery and release at sites of infection. Antimicrob. Agents Chemother. **33:**277–282.
20. **Gladue, R. P., and M. E. Snider.** 1990. Intracellular accumulation

of azithromycin by cultured human fibroblasts. Antimicrob. Agents Chemother. **34**:1056–1060.
21. **Hopkins, S.** 1991. Clinical toleration and safety of azithromycin. Am. J. Med. **91**(Suppl. 3A):40–45.
22. **Kees, F., H. Grobecker, J. B. Fourtillan, D. Tremblay, and B. Saint-Salvi.** 1988. Comparative pharmocokinetics of single dose roxithromycin versus erythromycin stearate in healthy volunteers. Br. J. Clin. Prac. **42**(Suppl. 55):51.
23. **Kirst, H. A., and G. D. Sides.** 1989. New directions for macrolide antibiotics: structural modifications and in vitro activity. Antimicrob. Agents Chemother. **33**:1413–1418.
24. **Peters, D. H., H. A. Friedel, and D. McTavish.** 1992. Azithromycin: a review of its antimicrobial activity, pharmacokinetic properties and clinical efficacy. Drugs **44**:750–799.
25. **Retsema, J., A. Girard, W. Schelkly, M. Manousos, M. Anderson, G. Bright, R. Borovoy, L. Brennan, and R. Mason.** 1987. Spectrum and mode of action of azithromycin (CP-62,993), a new 15-membered-ring macrolide with improved potency against gram-negative organisms. Antimicrob. Agents Chemother. **31**:1939–1947.
26. **Simon, H. J., and E. J. Yin.** 1970. Microbioassay of antimicrobial agents. Appl. Microbiol. **19**:573–579.
27. **Snedecor, G. W., and W. G. Cochran.** 1980. Statistical methods, 7th ed., p. 41. Iowa State University Press, Ames.
28. **Steinman, R. M., S. E. Brodie, and Z. A. Cohn.** 1976. Membrane flow during pinocytosis. J. Cell Biol. **68**:665–687.
29. **Vallee, E., E. Azoulay-Dupuis, J. J. Pocidalo, and E. Bergogne-Berezin.** 1992. Activity and local delivery of azithromycin in a mouse model of *Haemophilus influenzae* lung infection. Antimicrob. Agents Chemother. **36**:1412–1417.
30. **Wildfeuer, A., H. Laufen, D. Müller-Wening, and O. Haferkamp.** 1989. Interaction of azithromycin and human phagocytic cells. Uptake of the antibiotic and the effect on the survival of ingested bacteria in phagocytes. Arzneimittelforschung **39**:755–758.
31. **Williams, J. D.** 1991. Spectrum of activity of azithromycin. Eur. J. Clin. Microbiol. Infect. Dis. **10**:813–820.