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In Vitro and In Vivo Uptake of Azithromycin (CP-62,993) by Phagocytic Cells: Possible Mechanism of Delivery and Release at Sites of Infection

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Azithromycin, a novel azalide antibiotic, concentrated in human and mouse polymorphonuclear leukocytes (PMNs), murine peritoneal macrophages, and mouse and rat alveolar macrophages, attaining intracellular concentrations up to 226 times the external concentration in vitro. In murine peritoneal macrophages, azithromycin achieved concentration gradients (internal to external) up to 26 times higher than erythromycin. The cellular uptake of azithromycin was dependent on temperature, viability, and pH and was decreased by 2,4-dinitrophenol. Azithromycin did not decrease phagocyte-mediated bactericidal activity or affect PMN or macrophage oxidative burst activity (H_2O_2 release or Nitro Blue Tetrazolium reduction, respectively). Azithromycin remained in cells for several hours, even after extracellular drug was removed. However, its release was significantly enhanced by phagocytosis of *Staphylococcus aureus* (82 versus 23% by 1.5 h). In vivo, 0.05 μg of azithromycin was found in peritoneal fluids of mice 20 h after oral treatment with a dose of 50 mg/kg. Following caseinate-induced PMN infiltration, there was a sixfold increase in peritoneal cavity azithromycin to 0.32 μg, most of which was intracellular. Therefore, the uptake, transport, and later release of azithromycin by these cells demonstrate that phagocytes may deliver active drug to sites of infection.

The ability of an antimicrobial agent to penetrate into phagocytic cells is essential for activity against facultative intracellular organisms (11). However, the potential benefits attained from achieving high intraphagocytic concentrations are uncertain since intracellular bactericidal activity may not be proportionally enhanced (18). In fact, in some instances, a decrease in potential phagocyte-mediated bacterial killing mechanisms (O_2^- and H_2O_2) have been reported (13). On the other hand, one potential advantage of intracellular concentration with important implications for in vivo antibacterial efficacy is the targeted delivery of active drug to infection sites by the phagocyte. This was illustrated by Deysine et al. (4), who loaded leukocytes in vitro with polyacrylamide beads containing kanamycin and found that these cells, when injected in vivo, could carry the antibiotic to sites of infection. Even though in vitro loading of phagocytes is clinically impractical, this observation suggests that antibiotics with the ability to concentrate and be retained by cells in vivo could act in concert with the host immune system and be delivered to specific sites as part of the normal host cellular response to infection. Since efflux of antibiotics from cells has been demonstrated in vitro (2, 7), release of an antibiotic from phagocytes at these sites could produce locally high concentrations of active drug. Therefore, the ability of an antimicrobial agent to concentrate in phagocytes may be important for activity against extracellular, as well as intracellular, bacteria at localized areas of infection.

Azithromycin (CP-62,993; also designated XZ-450 [Pliva Pharmaceutical, Zagreb, Yugoslavia]) is a novel azalide antibiotic with improved in vivo potency, compared with that of erythromycin, against localized soft tissue infections (5). It differs from erythromycin in that it has a 15-membered ring (not 14) and contains two, rather than one, basic amine groups (1). This modification results in significant changes in pharmacokinetics (i.e., greater tissue penetration and longer elimination half-life) (5) and spectrum (i.e., potency against gram-negative bacteria) (16). Azithromycin has also been reported to have activity against intracellular pathogens (16, 20). Therefore, we examined the ability of azithromycin to enter and concentrate in phagocytic cells. In addition, the ability of phagocytes to retain, deliver, and potentially release azithromycin at infection sites was examined. The effect of azithromycin on normal phagocytic bactericidal mechanisms was also assessed.

MATERIALS AND METHODS

Animals. C3H/HeN male mice (15 to 18 g) and inbred Fisher 344 male rats (200 to 225 g) were purchased from Charles River Breeding Laboratories, Inc., Raleigh, N.C., and Kingston, N.Y., respectively.

Antibiotics. Radiolabeled azithromycin (9-deoxo-9a-aza-9a-[^{14}C]methyl-9a-homoerythromycin A) was prepared by methylation at the 9a-aza site of the precursor 9-deoxo-9a-aza-9a-homoerythromycin A by a modified Clarke-Eschweiler procedure, using radiolabeled aqueous formaldehyde (Dupont, NEN Research Products, Boston, Mass.). The radiolabeled drug was determined to have a radiopurity of >97% and a specific activity of 15.4 mCi/mmol. Unlabeled azithromycin was prepared by Pfizer Medicinal Chemistry Laboratories (1). The radiolabeled erythromycin (*N*-[^{14}C]methylerythromycin; Dupont, NEN) had a radiopurity of >95% and a specific activity of 54.3 mCi/mmol. Unlabeled erythromycin was purchased from Abbott Laboratories, North Chicago, Ill. The bioactivities of the radiolabeled antibiotics were verified by comparing their MICs with those obtained with the corresponding unlabeled antibiotic against a clinical isolate of *Staphylococcus aureus* (16).

Chemicals. 2,4-Dinitrophenol (DNP) and acridine orange were obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis. Nitro Blue Tetrazolium (NBT) and phorbol myristate acetate (PMA) were obtained from Sigma Chemical Co., St. Louis, Mo.

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Cell collection and volume determinations. The following cells were collected by standard techniques: human polymorphonuclear leukocytes (PMNs) (12), murine and rat alveolar macrophages (9), and murine resident peritoneal macrophages (6). Murine elicited PMNs were collected from the peritoneal cavity 18 h after injection of 3% sterile sodium caseinate. Cells were collected in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum and 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (GIBCO) buffer (RPMI medium).

Cell volume was determined by measuring the volume displaced by sedimented cells, using a Rannin micropipette. For macrophages, the volume of nonadherent cells was subtracted from the total volume. PMNs were found to have a volume of 2 μl/10^7 cells, which is similar to that found by Koga (10). Macrophages were found to have a volume of 10.9 μl/10^7 cells, which is higher than that calculated by Steinman et al. (19), who used 24-h adherent cells rather than freshly collected cells. More importantly, the internal concentration/external concentration (I/E) ratios obtained with erythromycin (used for comparison) were similar to those reported previously (2, 7, 9, 12).

Procedure for determining uptake of radiolabeled antibiotic. Quadruplicate samples containing 10^6 cells in RPMI medium were incubated in the presence of 10 μg of radiolabeled antibiotic per ml unless otherwise stated. At each time point, the cells were washed four times to remove extracellular antibiotic. PMNs in 96-well tissue culture plates were washed by centrifugation in cold Hanks balanced salt solution, while adherent macrophages in 24-well tissue culture plates were washed with warm Hanks balanced salt solution by decanting. ^{14}C-labeled antibiotic was also added to a sample of cells in each experiment immediately prior to washing to determine the amount of background noninternalized radiolabeled drug, which was then subtracted from all counts. The cells were then lysed (0.05% Triton X-100; Sigma), and the amount of radioactivity was determined in a liquid scintillation counter. The concentration of antibiotic was calculated from a standard curve. In addition, the concentrations of azithromycin in selected samples of cell lysates were also determined by an agar diffusion bioassay, using *Micrococcus luteus* (5). Protein concentrations (Coomassie blue; Bio-Rad Laboratories, Richmond, Calif.) were also measured in cell lysates to ensure cell number continuity between sample wells. The amount of cell-associated antibiotic per 10^7 cells was determined, and the I/E ratio was calculated based on the cell volume measurements determined as described above. Since the preparation of elicited murine PMNs was found to contain approximately 8% macrophages, the amount of antibiotic associated with murine peritoneal macrophages at the indicated times (determined from separate macrophage uptake experiments) was subtracted from the amount associated with the elicited cells.

Determination of oxidative burst activity. The oxidative burst activity of murine PMNs was determined by measuring H_2O_2 production (14). Cells were exposed to various concentrations of azithromycin for 2 h, washed, and suspended in phenol red-horseradish peroxidase medium which contained additional azithromycin equivalent to the initial concentration. The cells were warmed to 37°C for 30 min. PMA was then added to stimulate an oxidative burst.

Macrophage oxidative activity was determined by NBT reduction (17). Cells were cultured for 24 h in flat-bottom, 96-well tissue culture plates with and without azithromycin. NBT was added to each sample well, and the cells were stimulated with either PMA or latex (opsonized in fresh mouse serum for 30 min at 37°C).

In vitro macrophage bactericidal activity and bioactivity of released azithromycin. Macrophages were incubated with and without azithromycin in eight-chamber tissue culture chamber/slides (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.) and in 24-well tissue culture plates. After 24 h, the macrophages were washed to remove the extracellular azithromycin, and then opsonized *S. aureus* was added to each well. At 1, 6, and 24 h after the addition of *S. aureus*, adherent macrophages were stained with acridine orange (21) and examined by fluorescence microscopy for viable (green) and dead (red) intracellular bacteria. The total number of viable bacteria (intra- and extracellular) was determined (by the spread plate method) in the samples in 24-well tissue culture plates after lysing the macrophages by freeze-thawing.

In vivo uptake and delivery of azithromycin by phagocytic cells. In the first experiment, mice were treated with 50 mg of [^{14}C]azithromycin per kg by oral gavage immediately followed by an intraperitoneal injection of 1 ml of 3% sodium caseinate. Peritoneal cavities were then lavaged at 12 and 20 h after caseinate injection, times when PMNs were found to infiltrate into this area. In a separate experiment, animals were treated with azithromycin (orally) at the time of and 1 day after caseinate injection, and the peritoneal cavity was examined 4 days later (found to be optimal for macrophage infiltration). Control animals treated with azithromycin but not with caseinate were lavaged at the same times. Cells in the peritoneal lavage fluid were enumerated and differentiated (6). Each sample of lavage material was then centrifuged, and the amounts of azithromycin in the supernatant and in the cell pellet (cell associated) were determined as described above. Antibiotic concentration and bioactivity were confirmed by bioassay (5).

Statistical analysis. Statistical analysis was done with the Student *t* test. A *P* value of less than 0.05 was considered significant. All data are expressed as means ± 1 standard deviation.

RESULTS

Uptake of azithromycin by phagocytic cells. Azithromycin readily concentrated within PMNs and macrophages. After 2 h of incubation, azithromycin achieved an I/E ratio of 79 in human PMNs and 39 in murine PMNs (Table 1). The differential uptake of azithromycin and erythromycin in PMNs was approximately 4 to 1. In mouse or rat alveolar macrophages, the uptake of azithromycin was at least five times higher than that of erythromycin. However, the largest differential between azithromycin and erythromycin uptake was observed with murine peritoneal macrophages, in which azithromycin concentrated 15 times more than erythromycin after 2 h of incubation (I/E ratio for azithromycin = 62, I/E ratio for erythromycin = 4).

Azithromycin uptake continued for at least 24 h, achieving an I/E ratio in human PMNs of 226 and an I/E ratio in murine peritoneal macrophages of approximately 110 (Fig. 1). In contrast, the uptake of erythromycin by either cell type was essential complete within the first 30 min. At 24 h, azithromycin was concentrated 10-fold higher than erythromycin in human PMNs and 26-fold higher in murine peritoneal macrophages. Also, the amount of azithromycin that concentrated within peritoneal macrophages was directly proportional to the extracellular concentration (Fig. 2). In contrast,

TABLE 1. Uptake of azithromycin and erythromycin by various phagocytic cells

Cell type	Antibiotic[a]	Differential[b]	Antibiotic uptake	
			I/E	μg/10⁷ cells
Human PMNs	Azithromycin	4.9	79	1.58
	Erythromycin		16	0.32
Murine PMNs	Azithromycin	3.9	39	0.78
	Erythromycin		10	0.20
Murine alveolar macrophages	Azithromycin	5.9	170	18.66
	Erythromycin		29	3.18
Rat alveolar macrophages	Azithromycin	5.5	60	6.58
	Erythromycin		11	1.21
Murine resident peritoneal macrophages	Azithromycin	15.5	62	6.81
	Erythromycin		4	0.43

[a] Cells were incubated for 2 h with 10 μg of the antibiotic per ml.
[b] Ratio of azithromycin uptake to erythromycin uptake. All values are statistically significant.

the concentration gradient (I/E ratio) was constant for the concentrations evaluated (1 to 100 μg/ml).

Further experiments were done in an attempt to determine the mechanism(s) of azithromycin uptake by peritoneal macrophages. Uptake of azithromycin by phagocytes was prevented when cells were incubated at 4°C or after pretreatment of cells with formaldehyde (10% in phosphate buffer). Preincubating macrophages for 30 min in 1.0 mM DNP reduced the intracellular concentration of azithromycin by 32%. No effect on uptake was observed with 100 μM DNP. Uptake of azithromycin was not dependent upon the presence of serum over a range of 0 to 40%. Uptake was prevented, however, when cells were incubated at an acid pH (6.0) but was not significantly altered by incubation at an alkaline pH (8.4).

Since the uptake of some antibiotics has been reported to be mediated by membrane carrier systems (7, 8), the effects of membrane stimulants were also examined. Preincubation of macrophages with *S. aureus* (opsonized with fresh mouse serum for 1 h at 37°C) at a ratio of 1:40 reduced, but did not prevent, uptake of azithromycin (Table 2). In contrast, preexposure to PMA had no effect.

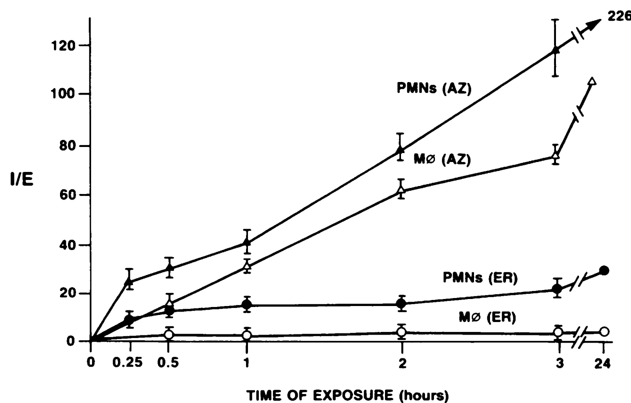

FIG. 1. Uptake of azithromycin (AZ) and erythromycin (ER) by human PMNs and murine peritoneal macrophages (Mϕ). The differential uptake between azithromycin and erythromycin was 10 to 1 for human PMNs and 26 to 1 for murine peritoneal macrophages after 24 h of incubation.

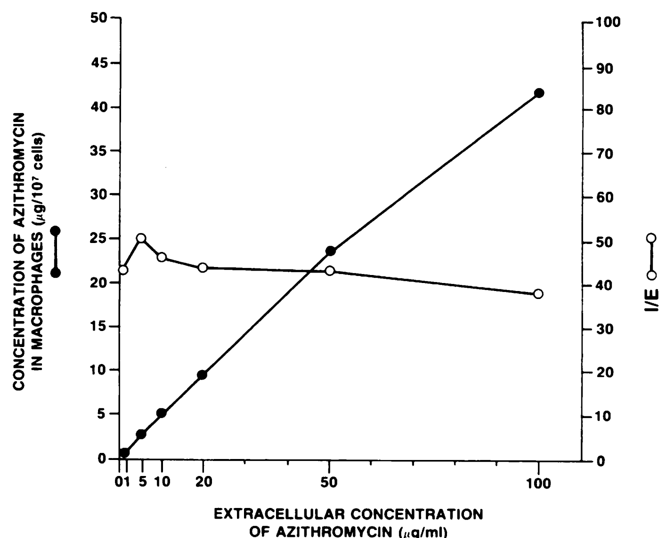

FIG. 2. Effect of extracellular concentration on uptake of azithromycin by resident peritoneal macrophages. Macrophages were incubated with the indicated concentration of azithromycin for 1 h.

Release of azithromycin from macrophages. Azithromycin was released slowly from macrophages after the removal of extracellular drug. After 1 h, only 19% of the azithromycin was released into the extracellular medium (Fig. 3). The release of azithromycin continued for 24 h. At this time, 93.3% was released. Thus, 6.7% of the initial amount remained cell associated (I/E ratio at this time was 85). In contrast, 1 h after the removal of extracellular antibiotic, 85% of the erythromycin had egressed from the cells, and by 3 h essentially all of the erythromycin had been released. In view of the relatively long half-life of azithromycin in cells, the effects of membrane stimulants on release were determined. Phagocytosis of opsonized *S. aureus* (40 cells per macrophage) significantly enhanced the release of azithromycin (Fig. 4). In contrast, PMA neither enhanced nor diminished release.

PMN and macrophage oxidative burst activity. Even though PMNs and macrophages concentrated azithromycin, no suppression of H_2O_2 release or NBT reduction was observed when cells were exposed to 50 μg of azithromycin per ml and then stimulated with PMA (Table 3). Similar results were observed when cells were exposed to concentrations of azithromycin as low as 0.05 μg/ml or when

TABLE 2. Effect of stimuli on uptake of azithromycin by murine resident peritoneal macrophages

Stimulus[a]	Uptake[b]	
	μg/10⁷ cells	I/E
None (control)	2.87 ± 0.22	25.1 ± 1.9
S. aureus	1.71 ± 0.20*	14.6 ± 1.5*
PMA	2.54 ± 0.22	21.5 ± 1.9

[a] Macrophages were exposed to the stimulus for 30 min prior to the addition of azithromycin.
[b] Means ± standard deviations determined after 45 min of incubation. *, Significant compared with the value for the control.

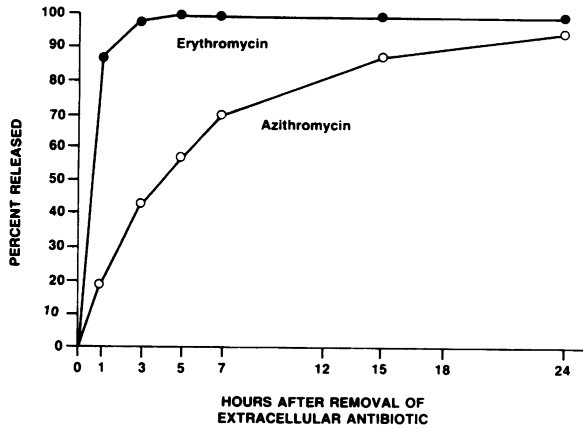

FIG. 3. Release of azithromycin and erythromycin from mouse peritoneal macrophages. Cells were labeled with the antibiotic for 24 h before being washed. The percent released is based on the amount of antibiotic in representative cells determined at time zero.

opsonized latex, rather than PMA, was used as the trigger for oxidative burst activity.

In vitro macrophage bactericidal activity and bioactivity of released azithromycin. When macrophages were incubated with *S. aureus* and then stained with acridine orange, the total number of bacteria ingested was the same whether macrophages were preexposed to azithromycin (10 μg/ml) or not. Intracellular killing ability was also not significantly different between macrophages exposed to azithromycin and controls (15 versus 18% at 1 h and 65 versus 70% at 6 h). Since azithromycin is bacteriostatic (MBC, 5.0 μg/ml; MIC, 0.15 μg/ml), this experiment demonstrated that normal phagocytic killing mechanisms remained intact.

In the next experiment, the numbers of viable intra- and extracellular bacterial cells were determined. Wells containing macrophages not loaded with azithromycin had increases in bacterial numbers (CFU) from 6×10^5/ml at 1 h to 2×10^8/ml at 6 h and 37×10^9/ml at 24 h. In contrast, wells containing macrophages preexposed to azithromycin (free azithromycin was washed out prior to the addition of bacteria) had no increase in bacterial numbers at 6 h, and in fact, bacterial numbers decreased after 24 h, resulting in $<10^2$ CFU/ml. If all the intracellular azithromycin had been released from the macrophages, the concentration of azithromycin in the extracellular fluid should have been approximately 1.09 μg/ml, a value above the MIC but below the MBC.

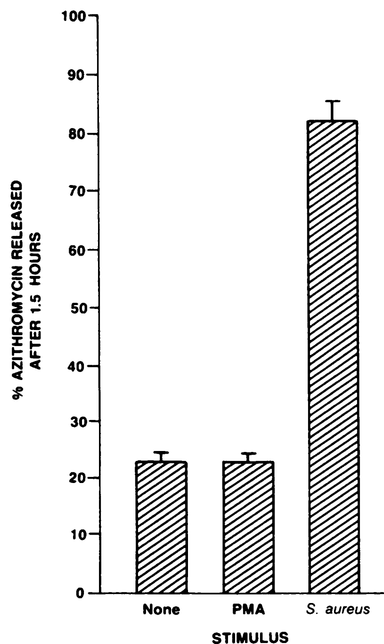

FIG. 4. Effect of membrane stimuli on release of azithromycin from macrophages. Cells were loaded with azithromycin for 24 h, washed, and then incubated with or without a stimulus for 1.5 h.

TABLE 3. Effect of azithromycin on PMN and macrophage oxidative burst activity

Cell type	Treatment[a]	Oxidative burst activity[b]
Murine PMNs	Azithromycin	5.26 ± 0.4
	Control	5.85 ± 0.4
Macrophages	Azithromycin	2.84 ± 0.6
	Control	3.00 ± 0.2

[a] Cells were exposed to 50 μg of azithromycin per ml for 2 h (PMNs) or 24 h (macrophages) prior to stimulation with PMA.
[b] Means ± standard deviations for eight samples in two separate experiments. Values are micromoles of H_2O_2 released for PMNs and micrograms of NBT reduced for macrophages.

In vivo uptake and delivery of azithromycin by phagocytic cells. The delivery of azithromycin to the peritoneal cavity was examined in peritoneal lavage fluid after oral treatment of mice with 50 mg of [^{14}C]azithromycin per kg. Initially, the azithromycin concentration in the peritoneal cavity increased in caseinate-treated animals in direct proportion to the number of PMNs migrating into this site (Table 4). By 20 h after caseinate injection, the total azithromycin in the peritoneal cavity increased sixfold (75% cell associated; intracellular concentration, 79 μg/ml). This increase in azithromycin did not appear to be a result of perturbation of the blood-peritoneum boundary since (i) essentially no erythrocytes were observed in the lavage fluid, (ii) most of the azithromycin was found in the leukocyte pellet (and not free within the fluid), and (iii) azithromycin increased over time as more PMNs migrated into this site.

Delivery of azithromycin to the peritoneal cavity by monocytes was also examined. Animals were treated twice

TABLE 4. Effect of phagocyte infiltration on concentration of azithromycin in peritoneal fluids

Time[a]	No caseinate			Caseinate		
	PMNs (10^6)	Macrophages (10^6)	Total peritoneal azithromycin (μg)	PMNs (10^6)	Macrophages (10^6)	Total peritoneal azithromycin (μg)[b]
12 h[c]	0	1.2	0.05	6.5	1.2	0.122
20 h[c]	0	1.2	0.05	15.0	1.2	0.322
4 days[d]	0	1.2	ND[e]	0.5	6.5	0.220

[a] Time after caseinate injection. There were three animals per group for each time and treatment.
[b] The relative amounts of peritoneal azithromycin found in the leukocyte pellet of caseinate-treated animals were 52% at 12 h, 75% at 20 h, and 92% at 4 days.
[c] Azithromycin (50 mg/kg) was administered immediately prior to caseinate injection.
[d] Azithromycin (50 mg/kg) was administered at the time of and 24 h after caseinate injection.
[e] ND, None detected.

with azithromycin, once at the time of caseinate administration and again 24 h later. Four days after caseinate injection, macrophage numbers increased 5.4-fold (Table 4). Again, essentially no erythrocytes were observed in the peritoneal cavity. Whereas azithromycin was undetectable at this time in non-caseinate-treated animals, 0.22 μg of azithromycin was present in peritoneal fluids of animals injected with caseinate (92% cell associated; intracellular concentration, 32 μg/ml). This increase in peritoneal azithromycin occurred at a time when levels in serum were undetectable (data not shown).

DISCUSSION

These results demonstrate that azithromycin was concentrated in phagocytic cells. For example, azithromycin achieved intracellular concentrations 226 times greater than the extracellular concentration in human PMNs, achieving a final concentration of 4.52 μg/10^7 cells. This translates to an intracellular concentration of 2.26 mg of azithromycin per ml. While erythromycin concentrated in phagocytes (Table 1 and Fig. 1) (2, 7, 9, 12), the magnitude was considerably less than that for azithromycin. Furthermore, the time courses of uptake were different for the two antibiotics. While the uptake of erythromycin was essentially complete within 30 min, azithromycin continued to be taken up over a 24-h period. The magnitude of intracellular concentration achieved over longer time periods might have relevance since azithromycin is reported to have long half-lives in tissue and serum (5), which may expose phagocytes, as well as other cells, to the drug for extended periods of time. The ability of azithromycin to penetrate and concentrate in phagocytes may explain why azithromycin is effective against intracellular pathogens, including *Listeria monocytogenes* and *Chlamydia trachomatis* (5, 16, 20).

The uptake and release of azithromycin by phagocytic cells may provide a unique means of delivering azithromycin to sites of infection. Azithromycin not only concentrated in phagocytes (in vivo and in vitro) but was also maintained for a relatively long period of time even in the absence of extracellular antibiotic. This suggests that, in vivo, phagocytic cells could retain azithromycin even though levels in serum may be negligible. In addition, phagocytic cells transported intracellular azithromycin to a localized site in response to caseinate stimulation. Such elicitation of phagocytic cells also occurs as part of the normal host cellular response to infection. Furthermore, the rapid release of azithromycin in the presence of bacteria, demonstrated in vitro, may produce locally high concentrations of active drug. Since normal phagocytic killing mechanisms appeared intact in azithromycin-loaded cells, the released azithromycin (shown to be bioactive) may act together with normal phagocytic bactericidal mechanisms to help eradicate intra- and extracellular infections caused by sensitive organisms. Thus, intracellular concentrations of azithromycin in peripheral blood and tissue may be more relevant to in vivo anti-infective activity than the total levels in serum or plasma, which are classically used for predicting antibacterial efficacy. Along these lines, in vivo antibacterial efficacy was observed in animals treated with azithromycin at times when levels in serum were undetectable (A. E. Girard, D. Girard, J. A. Retsema, and R. M. Shepard, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 785, 1988).

Exposure of phagocytes to *S. aureus* enhanced the release of azithromycin from cells (Fig. 4) and reduced its in vitro concentrating ability. Reduced uptake during in vitro phagocytosis has also been reported for roxithromycin and erythromycin (7), whereas the opposite has been reported for clindamycin (18). The uptake of antibiotics by phagocytes is important. However, it would not be beneficial if all the antibiotic entered the phagocyte in the presence of extracellular bacteria (e.g., at infection sites). Also, even though azithromycin uptake was decreased by *S. aureus*, it was not prevented. The cause for this decreased uptake of azithromycin in the presence of bacteria might relate to the enhanced release shown to occur following phagocytosis, thus altering the equilibrium in favor of decreased uptake.

The relative contribution of active versus passive transport in the uptake of antibiotics is difficult to determine. It is conceivable that both processes could be involved in the uptake of azithromycin. The metabolic inhibitior DNP inhibited azithromycin uptake, suggesting active transport is involved. However, only a high concentration (1 mM) was effective, and even then only 32% inhibition was observed compared with controls. Passive transport processes are also suggested by the direct relationship observed between extracellular concentration and intracellular accumulation and by the inhibition of uptake by low temperature or fixation, either of which could decrease membrane fluidity and thus prevent diffusion. Although the extent of uptake was greater than usual for a passive transport process, one mechanism by which diffusion may lead to intracellular accumulation might relate to the tendency of basic compounds to be lysosomotropic and to become trapped in lysosomes as a result of the acidic pH (3, 15). The presence of two basic amine groups in the structure of azithromycin (1) may allow for greater ionization and trapping than that which may occur with classical macrolides, like erythromycin, which contain only one basic amine group. Along these lines, acidity did inhibit diffusion of azithromycin into cells. The partial inhibition of uptake by DNP might therefore be explained by inhibition of proton pump activity, which is required to maintain lysosomal pH (3), thus leading to more extensive uptake and trapping.

In summary, azithromycin was shown in vitro and in vivo to concentrate in phagocytic cells. The presence of increased levels of azithromycin in the peritoneal cavities of stimulated mice suggests the potential for phagocytes to deliver azithromycin to sites of infection. This, coupled with the enhanced release of azithromycin from macrophages in the presence of bacteria, further suggests that azithromycin, in concert with the host immune system, is a directed, perhaps sustained-release, antimicrobial agent at infection sites.

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