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In Vitro Demonstration of Transport and Delivery of Antibiotics by Polymorphonuclear Leukocytes

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Several antibiotics are concentrated inside polymorphonuclear leukocytes (PMN). To investigate whether PMN could act as vehicles for delivery of antibiotics, we combined an assay measuring PMN chemotaxis under agarose with a bioassay measuring levels of antibiotic in agar. Double-layer plates were made by pouring a layer of chemotaxis agarose into tissue culture plates and then adding a thin layer of Trypticase soy agar. Neutrophils were incubated with antibiotic for 1 h and then were washed and placed in wells made in the plates. After allowing PMN to migrate under the agar toward a chemoattractant well containing formyl-methionine-leucine-phenylalanine for 3 h, *Streptococcus pyogenes* was streaked on top of the agar and grown overnight. PMN migration and zones of inhibition of bacterial growth were measured. Neutrophils migrated 2.51 ± 0.16 mm toward the chemoattractant well and 1.48 ± 0.12 mm toward the medium well; migration was not significantly affected by any of the antibiotics used. Plates with PMN incubated without antibiotic showed insignificant inhibition of bacterial growth toward chemoattractant and medium wells (0.38 ± 0.18 and 0.14 ± 0.12 mm, respectively; for both, $P > 0.05$ for difference from 0). PMN incubated with oxacillin (3 μg/ml), a drug not concentrated in PMN, caused a similar lack of inhibition (0.28 ± 0.09 mm toward chemoattractant; 0.14 ± 0.03 mm toward medium). Incubation with 30 μg of ciprofloxacin per ml resulted in inhibition that was similar in both directions (1.40 ± 0.16 versus 1.18 ± 0.13 mm). However, for PMN incubated with azithromycin (3 μg/ml), an agent highly concentrated inside phagocytes, there was a large degree of inhibition which was significantly greater in the direction of chemoattractant than in the direction of medium (3.47 ± 0.30 versus 1.89 ± 0.25 mm; $P < 0.001$), indicating that release of bioactive azithromycin by neutrophils occurred after migration. Likewise, after incubation with rifampin (10 μg/ml), which is also concentrated by PMN, inhibition was significantly greater in the direction of chemoattractant than in the direction of medium (1.54 ± 0.24 versus 0.81 ± 0.28 mm; $P = 0.001$). We conclude that for certain antibiotics, PMN may act as vehicles for transport and delivery of active drug to sites of infection.

Several antibiotics are known to be concentrated inside phagocytes. Among these are lipid-soluble agents such as rifampin and weak bases such as erythromycin (11). A striking example of this phenomenon is azithromycin, an azalide structurally related to erythromycin, which achieves intracellular levels over 200 times greater than extracellular levels (5). Intracellular penetration is considered a necessary (though not sufficient) property for activity against intracellular pathogens (6). In addition, this property may have relevance for treatment of extracellular infections. Polymorphonuclear leukocytes (PMN) migrate from the bloodstream through tissues to sites of infection. If these cells could effectively carry antibiotic along with them and release it at the site of infection, this would provide a means of achieving locally higher levels exactly where antibiotic is most needed. In order for neutrophils to act as effective vehicles for transport and delivery of a drug, a number of requirements must be met: the drug must be highly concentrated inside the cell, and these high levels must be maintained during migration of the phagocyte; the drug must not adversely affect normal migration and chemotaxis; and at a later point, the drug must be released from the cell and maintain its activity against microorganisms.

We developed an in vitro model to evaluate antibiotics for these properties by combining an assay measuring PMN chemotaxis under agarose (10) with a modified bioassay for measuring levels of antibiotic (12). Double-layer agar plates were made with a bottom layer of chemotaxis agar and a very thin top layer of Trypticase soy agar. After incubation with antibiotic, neutrophils were washed to remove extracellular antibiotic and then were allowed to migrate under the bottom agar layer toward a chemoattractant. Bacteria were inoculated onto the top agar layer. The release of antibiotic by the PMN could then be assessed by measuring the zone of inhibition of bacterial growth. *Streptococcus pyogenes* was used as the assay organism because it was sensitive to all of the antibiotics tested and showed negligible inhibition by neutrophils alone. The assay was performed with an antibiotic which does not accumulate intracellularly (oxacillin [13]) and several which do (azithromycin [5], rifampin [6, 7, 11], and ciprofloxacin [1, 13]).

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MATERIALS AND METHODS

Determination of MICs. MICs of antibiotics for the organism used in the assays were determined by the broth dilution method (8).

Antibiotics. Azithromycin was provided by Pfizer Pharmaceuticals, New York, N.Y. Ciprofloxacin was provided by Miles Pharmaceuticals, West Haven, Conn. Rifampin and

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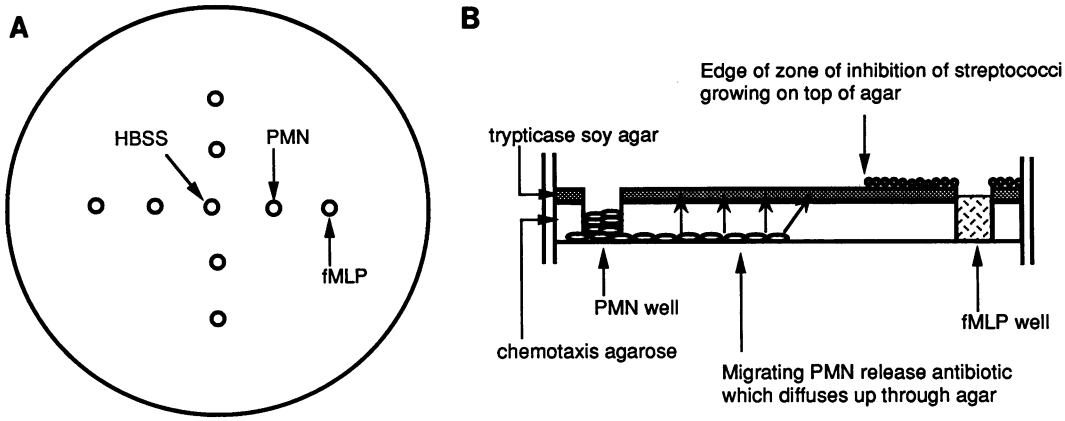

FIG. 1. Diagram of double-layer agar plates. (A) Top view to show arrangement of wells. (B) Detail of cross-section through one set of neutrophil and chemoattractant wells.

oxacillin were obtained from Sigma Chemical Company, St. Louis, Mo. A stock solution of azithromycin was made by initially dissolving azithromycin in ethanol and then diluting this with Hanks balanced salt solution (HBSS; Whittaker Bioproducts, Inc., Walkersville, Md.). Rifampin solution was made fresh daily by initially dissolving rifampin in methanol and diluting this further with HBSS. Stock solutions of ciprofloxacin and oxacillin were made in sterile water. All stock solutions were kept at −70°C until used.

Bacteria. *S. pyogenes* ATCC 12344 was kept on blood agar plates and subcultured every 3 days. For each experiment, a 6-h culture of the organism in Trypticase soy broth (Difco Laboratories, Detroit, Mich.) was made.

Isolation of PMN. Human PMN were obtained from normal donors and isolated from heparinized (10 U/ml) venous blood by a one-step Ficoll-Hypaque separation procedure (2). The cells were washed three times and then resuspended in HBSS.

Preparation of agar plates. Double-layer agar plates were made with a bottom layer consisting of chemotaxis agar and a top layer of Trypticase soy agar. Chemotaxis agar was made by combining the following (per 100 ml): 1.2 g of agarose (Litex type HSA; Accurate Chemical and Scientific Corp., Westbury, N.Y.), 10 ml of heat-inactivated pooled human serum, 10 ml of 10× concentrated minimal essential medium (HMEM; Whittaker Bioproducts), 80 ml of sterile water, 1.0 ml of 7.5% sodium bicarbonate, and 1.0 ml of 1 M HEPES buffer (final concentration, 10 mM; Sigma Chemical Co.). While hot, agar was pipetted (2.5 ml per plate) into tissue culture plates (60 by 15 mm). After the bottom layer had hardened, Trypticase soy agar (Difco Laboratories) was pipetted (1.0 ml per plate) on top. This gave a chemotaxis agar layer about 1 mm thick and a Trypticase soy agar layer about one-half mm thick. After both layers had hardened, 3-mm-diameter wells were cut in the agar 4 mm apart in the pattern shown in Fig. 1. Each plate contained one control sample (PMN incubated without antibiotic) and three samples of PMN incubated in different antibiotic concentrations.

Incubation of PMN with antibiotic and chemotaxis under agarose. PMN (5×10^6/ml) were incubated at 37°C with or without antibiotic for 60 min and then were washed to remove extracellular antibiotic. Washing consisted of two cycles of centrifuging at room temperature for 5 min at 150 × *g* and then immediately pouring off the supernatant and resuspending the cells in 1 ml of HBSS. The cells were then concentrated by centrifugation (again at room temperature for 5 min at 150 × *g*), and 8 μl of the cell pellet was placed in each of the middle wells in the agar plates. The cell pellets were typically about 25 μl in volume; thus, about 1.6×10^6 PMN were placed in each well. The total time elapsed between the end of incubation with antibiotic and placement of PMN in wells was about 20 min. The innermost well was filled with HBSS as control medium, and the outer wells were filled with 10^{-7} M formyl-methionine-leucine-phenylalanine (fMLP) as a chemoattractant. All plates were incubated at 37°C for 3 h to allow PMN to migrate. At that time, some of the plates were fixed with methanol, agar was fixed and removed with phosphate-buffered formalin, and the PMN were stained with Giemsa stain. Neutrophil migration toward chemoattractant and medium wells was measured under a microscope.

Assay of release of antibiotic. After the 3-h period for migration, the rest of the plates were inoculated by wire loop with a 6-h broth culture of *S. pyogenes* ATCC 12344 and incubated in a 37°C 5% CO_2 incubator overnight. The next morning, the zones of inhibition of bacterial growth in the directions of the chemoattractant and medium wells were measured under a microscope. Photographs of representative plates are shown in Fig. 2.

Effect of PMN migration on diffusion and activity of antibiotic. To see whether PMN alter movement of extracellular antibiotic by creating a pathway under the agar, the following procedure was performed. One well contained PMN incubated without antibiotic for 60 min and washed three times in HBSS as in the other studies. Midway through the 3-h migration period, 5 μl of 1-μg/ml oxacillin was added to the PMN well, and then the incubation continued. The second sample also consisted of PMN incubated without antibiotic, but halfway through the migration period, 5 μl of

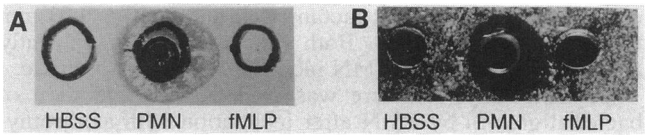

FIG. 2. Example of appearance of plates. (A) Plate after fixation and staining to show pattern of PMN migration. (B) Agar plate after overnight incubation to show zone of inhibition of bacterial growth created by PMN incubated with azithromycin. (For this example, *Micrococcus luteus* was used as the assay organism since its colonies are photographed more easily.)

HBSS, was added to this well. The third sample had no PMN. In this well, 8 μl of HBSS was initially placed and then 5 μl of 1-μg/ml oxacillin was added at the midpoint of the 3-h migration period. All samples had HBSS medium and fMLP chemoattractant wells. All plates were inoculated with *S. pyogenes* after the migration period and incubated overnight, and zones of inhibition of bacterial growth were determined as described above.

Inhibition of bacterial growth by azithromycin alone. Identical double-layer plates were used as in the other experiments. To assess the zone of inhibition resulting from azithromycin alone, an amount of antibiotic representing the estimated quantity associated with neutrophils was used (see Discussion). Eight microliters of 5-μg/ml azithromycin was placed in middle wells, and the plates were incubated for 3 h for the PMN plates, after which the surface of the agar was inoculated with *S. pyogenes* as described above. The resulting zone of inhibition was measured under a microscope.

Statistical analysis. All values are expressed as means ± standard errors of the means for five separate experiments with different PMN donors. For each experiment, three plates were used to measure PMN migration and four plates were used to measure zones of inhibition of bacterial growth. Statistical significance was assessed by using paired Student's *t* test with $P < 0.05$ as the level of significance.

RESULTS

MICs. The MICs of the various antibiotics for the strain of *S. pyogenes* used in the assays were as follows (in micrograms per milliliter): oxacillin, 0.06; azithromycin, 0.5; rifampin, 0.25; and ciprofloxacin, 0.25. The organism was sensitive to all antibiotics tested.

PMN migration. PMN migrated 2.51 ± 0.16 mm toward the chemoattractant well and 1.48 ± 0.12 mm toward the medium well, demonstrating good chemotaxis ($P < 0.001$ for the difference between directed and nondirected migration). Neither directed nor nondirected migration was affected by any of the antibiotics at the concentrations used.

Inhibition of bacterial growth by PMN alone. PMN incubated without antibiotic did not produce any significant inhibition of bacterial growth. Measured inhibition by PMN alone was 0.38 ± 0.18 mm in the direction of the chemoattractant well and 0.14 ± 0.12 mm toward the medium well (for both values, $P > 0.05$ for the difference from 0). For PMN incubated in 0.003% ethanol or 0.3% methanol (corresponding to the highest concentrations in azithromycin and rifampin samples, respectively), inhibition was not different from that by PMN incubated in HBSS.

Inhibition of bacterial growth by PMN after incubation with antibiotics. **(i) Oxacillin.** Oxacillin is known to be excluded from PMN for the most part and as expected did not affect the ability of PMN to inhibit growth of *S. pyogenes*, even after incubation with 50 times the MIC. Inhibition by PMN incubated with 3 μg of oxacillin per ml was only 0.28 ± 0.09 mm toward the chemoattractant well and 0.14 ± 0.03 mm toward the medium well. Both values are not significantly different from those of PMN incubated without antibiotic.

(ii) Azithromycin. There was considerable inhibition of bacterial growth by PMN after incubation with azithromycin. Moreover, this inhibition was significantly greater in the direction of the chemoattractant well than in the direction of medium well. For PMN incubated with 3 μg of azithromycin per ml (six times the MIC), inhibition was 3.47 ± 0.30 mm in the direction of the chemoattractant and 1.89 ± 0.25 mm toward the medium well ($P < 0.001$). This relationship held true at lower concentrations as well, as shown in Fig. 3. Even for neutrophils incubated in 0.3 μg of azithromycin per ml, inhibition of bacterial growth was significantly greater than that by PMN incubated without antibiotic ($P = 0.005$) and significantly greater in the direction of chemoattractant than in the direction of medium ($P = 0.01$).

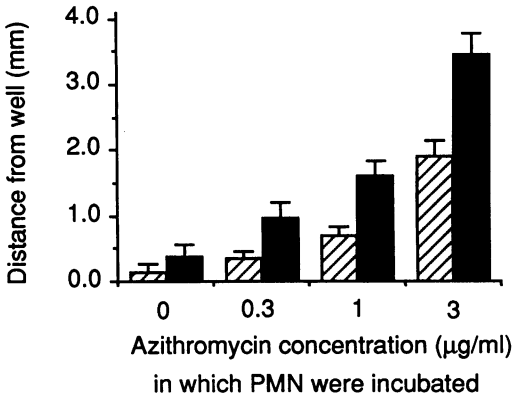

FIG. 3. Inhibition of streptococcal growth in the directions of chemoattractant (■) and medium (▨) by PMN after incubation with various concentrations of azithromycin.

(iii) Rifampin. Although rifampin was twice as active as azithromycin for *S. pyogenes* (MICs of 0.25 versus 0.5 μg/ml, respectively), incubation of neutrophils with the same concentrations of rifampin did not result in any significant inhibition (for 3 μg of rifampin per ml, 0.46 ± 0.12 mm toward chemoattractant and 0.12 ± 0.03 mm toward medium). When rifampin was used at higher concentrations, however, inhibition did occur and again was greater in the direction of chemotaxis. At 10 μg rifampin per ml (40 times the MIC), inhibition was 1.54 ± 0.24 mm toward chemoattractant and 0.81 ± 0.28 mm toward medium ($P = 0.001$), and at 30 μg/ml, values were 2.76 ± 0.70 and 1.61 ± 0.45 mm, respectively ($P = 0.017$).

(iv) Ciprofloxacin. For PMN incubated with concentrations of ciprofloxacin up to 10 μg/ml, inhibition of bacterial growth was not significantly greater than that by PMN alone. After incubation with 30 μg/ml, inhibition was noted. Unlike the results with azithromycin, however, inhibition in the direction of chemoattractant was not significantly different from that in the direction of medium (1.40 ± 0.16 versus 1.18 ± 0.13 mm, respectively; $P = 0.11$). Figure 4 compares the mean distance of PMN migration with the degree of inhibition of bacterial growth produced by PMN after incubation with the antibiotics. While the zone of inhibition after incubation with ciprofloxacin was smaller than the distance of PMN migration, incubation with azithromycin or rifampin resulted in inhibition that extended beyond the area of migration.

(v) Effect of PMN migration on antibiotic bioassay. Experiments were done to evaluate whether neutrophils migrating under agar might somehow affect the diffusion of antibiotic through the agar. In addition, any additive effect or interference by PMN or fMLP on antibiotic activity could be assessed. Oxacillin was used since it is not taken up by PMN. Bacterial inhibition zones created by PMN alone, oxacillin alone, and PMN plus oxacillin were compared by using the same bioassay used in the other experiments. PMN alone showed a lack of inhibition similar to that in the previous studies. Oxacillin alone produced a roughly circular

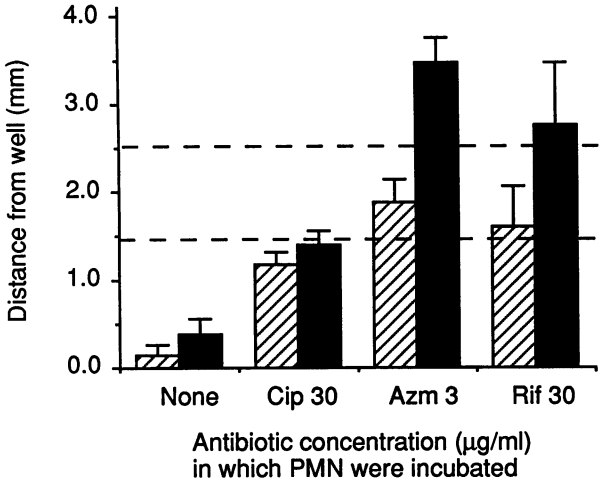

FIG. 4. Comparison of distance of PMN migration with size of zone of inhibition of bacterial growth resulting from incubation of PMN with various antibiotics. PMN migration was not affected by any of the antibiotics used. Inhibition of bacterial growth in the directions of chemoattractant (■) and medium (▨) in the presence of the following drugs is indicated: none, PMN incubated without antibiotic; Cip 30, 30 μg of ciprofloxacin per ml; Azm 3, 3 μg of azithromycin per ml; Rif 30, 30 μg of rifampin per ml. The top and bottom dashed lines represent PMN migration toward chemoattractant and toward medium, respectively.

zone of inhibition with the distance in the direction of the fMLP well (2.49 ± 0.21 mm) not significantly different from that in the direction of the HBSS well (2.40 ± 0.22 mm). When PMN and oxacillin were placed together in the well, inhibition was very similar (2.45 ± 0.22 mm toward fMLP, 2.40 ± 0.18 mm toward HBSS) to that of oxacillin alone. Thus, inhibition by the antibiotic was not affected by the presence of either the neutrophils or the fMLP used as a chemoattractant.

(vi) Inhibition of bacterial growth by azithromycin alone. The zone of inhibition noted with 8 μl of 5-μg/ml azithromycin (representing the estimated amount of drug associated with neutrophils; see Discussion) was 2.18 ± 0.28 mm toward the medium well and 2.23 ± 0.25 mm toward the chemoattractant well. These values are not significantly different from those for the zone of inhibition of bacterial growth in the direction of the medium well resulting from PMN incubated in 3 μg of azithromycin per ml (1.89 ± 0.25 mm). These values are substantially less than those for the inhibition of bacterial growth in the direction of the chemoattractant well seen with PMN incubated with 3 μg of azithromycin per ml (3.47 ± 0.30 mm; $P < 0.001$), providing further evidence that PMN are transporting the antibiotic.

DISCUSSION

The ability of neutrophils to migrate preferentially to sites of infection makes them attractive as a delivery mechanism for antibiotics. With the proper cellular pharmacokinetics, an antibiotic could be taken up by PMN which would then transport the drug and later release it. This may provide a means of attaining higher levels of antibiotic in tissues where the antibiotic is most needed. Our in vitro experiments showed that this type of transport and delivery by human neutrophils is possible.

Azithromycin is avidly taken up by neutrophils and has a long intracellular half-life. Gladue et al. found an intracellular-to-extracellular ratio of azithromycin in PMN of about 40 after 1 h of incubation; 1 h after removal of extracellular drug, macrophages still had 81% of their original intracellular concentration (5). As part of the same study, mice were given oral azithromycin, and azithromycin levels in peritoneal lavage fluid of caseinate-injected and noninjected mice were compared. At 20 h after the administration of the oral dose and the caseinate injection, the level of peritoneal azithromycin was higher in injected than in noninjected animals, and this coincided temporally with the influx of PMN into peritoneums of injected mice. The investigators did not exclude the possibility of inflammation increasing peritoneal antibiotic penetration independent of carriage by leukocytes. Girard et al. (4) dosed rats with azithromycin and then implanted discs containing *Staphylococcus aureus*. Concentrations of azithromycin in the discs increased as an inflammatory response to the discs developed (4). While it is not certain that the increased azithromycin concentrations were due to the arrival of PMN in either case, these studies suggested that possibility.

We developed a model to address this issue in vitro. After incubation with antibiotic, PMN migrate under agarose; inhibition of bacteria inoculated on top of the agar shows the location and concentration of bioactive drug released. Since PMN are washed to remove extracellular antibiotic, any antibiotic in the system must have been cell associated. Experiments with PMN incubated without any antibiotic demonstrated minimal inhibition by products of the neutrophils themselves. Since oxacillin does not accumulate intracellularly (intracellular-to-extracellular ratio of <0.1 at 20 μg/ml [13]), we expected no significant inhibition by PMN incubated with this antibiotic, and none was seen even after incubation with 50 times the MIC.

After incubation with azithromycin, however, substantial inhibition of bacterial growth was found. In addition, this inhibition was significantly greater in the direction of neutrophil chemotaxis. This demonstrates that release of bioactive azithromycin by PMN occurred after migration, since antibiotic released while the PMN were still in the well would produce equal inhibition in both directions. In fact, the inhibition seen followed a dose-response relationship, which correlates with the observation that intracellular azithromycin accumulation varies directly with extracellular concentration (5).

We performed another set of experiments to rule out a confounding effect of the chemoattractant or the neutrophils themselves on antibiotic diffusion and activity. Inhibition by oxacillin alone was compared with that by oxacillin and PMN together. The zone of inhibition for oxacillin alone was equidistant toward chemoattractant and medium wells, demonstrating no effect of the fMLP used as the chemoattractant. When the same amount of oxacillin was added to PMN, inhibition was again equal in both directions and equal to that caused by oxacillin alone, showing no influence of the PMN on antibiotic diffusion or activity. Another possible explanation for the results seen with azithromycin is that the inhibition of bacterial growth was not due to antibiotic per se but to increased release of neutrophil antimicrobial compounds. We feel that this is unlikely since neutrophils incubated without antibiotic (and already stimulated by fMLP) produced minimal inhibition of bacteria.

For comparison, we looked at the zone of inhibition of bacterial growth resulting from filling the sample well with azithromycin without PMN. The mass of antibiotic remaining in 8 μl of a PMN cell pellet after a 1-h incubation with

antibiotic, followed by washing, can be roughly estimated by the formula $(I/E) \times C_e \times 0.001$ ml $\times$ % retention $\times$ 32%, where I/E is the ratio of intracellular to extracellular concentrations after 1 h, C_e is the extracellular concentration in micrograms per milliliter, 0.001 ml is the volume of 5×10^6 PMN (5, 9), percent retention is the fraction remaining intracellular 20 min after removal of extracellular antibiotic, and 32% is the fraction of the cell pellet placed in the well (8 μl of the 25-μl pellet). For PMN incubated in 3 μg of azithromycin per ml, I/E is 40 and the percent retention is nearly 100% (5), so that the estimated mass of total intracellular azithromycin placed in each PMN well was 0.038 μg. We used 0.04 μg (8 μl of 5-μg/ml azithromycin) in the experiments determining zones of inhibition by azithromycin without PMN. The consequent zone of inhibition was similar to that seen with PMN incubated with azithromycin in the direction of the medium but was considerably smaller in the direction of the chemoattractant. These results suggest that release of antibiotic occurred after migration toward the chemoattractant.

Neutrophils incubated with rifampin also showed increased inhibition of bacterial growth, though much higher concentrations were required. While increased inhibition could be demonstrated for PMN incubated with as little as 0.3 μg of azithromycin per ml (less than the MIC), incubation with 10 μg of rifampin per ml (40 times the MIC) was required for a similar effect. This difference is probably in part a result of rifampin's lower intracellular concentration (two to nine times the extracellular concentration) and lower degree of intracellular retention after removal of extracellular antibiotic (6, 7, 11).

Inhibition was also seen after incubation with ciprofloxacin, though again this required high concentrations. In fact, significant inhibition was not seen until incubation with 30 μg/ml, which is 120 times the MIC. Furthermore, inhibition in the direction of chemoattractant was not significantly different from inhibition in the direction of medium, suggesting that release of drug by PMN occurred before migration. Not only is ciprofloxacin taken up to a lesser degree (two to five times the extracellular concentration) than azithromycin, but it also has much more rapid efflux from the cell after removal of extracellular antibiotic (1, 13). It may be, therefore, that ciprofloxacin was released from the PMN in our experiments before any substantial migration and that simple diffusion gave an inhibition zone that was more circular.

For both ciprofloxacin and rifampin, it is likely that a substantial amount of intracellular drug was lost during the washes to remove extracellular drug, in contrast to azithromycin, which has prolonged retention as stated above. Höger et al. found that rifampin rapidly eluted from PMN after one wash, reaching a concentration after 5 min of about 30% of that originally present; from the 5-min point on, rifampin efflux levelled off (7). Easmon and Crane reported rapid efflux of ciprofloxacin that continued for 30 min, at which point less than 20% of the original intracellular concentration remained (1). From the efflux curves presented in these two studies, we can approximate the percent retention of rifampin and ciprofloxacin after the 20 min elapsed for washing in our experiments. We can then use the above formula to estimate the amount of rifampin remaining in PMN after incubation with 10 μg/ml followed by washing (with an I/E of 9 and a retention of 25% [7]) and arrive at a value of 0.0072 μg. The same calculation for 30 μg ciprofloxacin per ml (with an I/E of 6 and a retention of 30% [1]) gives an estimate of 0.017 μg. These values are certainly lower than the estimate of the amount of azithromycin remaining after washing made above. Given this limitation created by the need to wash cells to remove extracellular antibiotic in our experiments, it may be that PMN transport of low concentrations of rifampin or ciprofloxacin can occur in vivo.

In summary, our in vitro experiments documented transport and delivery of bioactive drug by human neutrophils. This was particularly evident in the case of azithromycin, an agent which achieves very high intracellular concentrations and has prolonged intracellular retention. Uptake, transport, and release were shown with levels found in vivo (3). In fact, PMN delivered enough azithromycin to result in inhibition of bacterial growth even after the PMN had been incubated with sub-MICs. These results demonstrate that neutrophils may act as a mechanism for concentration, transport, and delivery of antimicrobial agents to sites of infection. Further work is needed to explore the therapeutic implications of these findings.

ACKNOWLEDGMENTS

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REFERENCES

1. **Easmon, C. S. F., and J. P. Crane.** 1985. Uptake of ciprofloxacin by human neutrophils. J. Antimicrob. Chemother. **16**:67–73.
2. **Ferrante, A., and Y. H. Thong.** 1980. Optimal conditions for simultaneous purification of mononuclear and polymorphonuclear leucocytes from human blood by the Hypaque-Ficoll method. J. Immunol. Methods **36**:109–117.
3. **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.
4. **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):S61–S72.
5. **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.
6. **Hand, W. L., and N. L. King-Thompson.** 1986. Contrasts between phagocyte antibiotic uptake and subsequent intracellular bactericidal activity. Antimicrob. Agents Chemother. **29**: 135–140.
7. **Höger, P. H., K. Vosbeck, R. Seger, and W. H. Hitzig.** 1985. Uptake, intracellular activity, and influence of rifampin on normal function of polymorphonuclear leukocytes. Antimicrob. Agents Chemother. **28**:667–674.
8. **Jones, R. N., A. L. Barry, T. L. Gavan, and J. A. Washington II.** 1985. Susceptibility tests: microdilution and macrodilution broth procedures, p. 972–977. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
9. **Koga, H.** 1987. High-performance liquid chromatography measurement of antimicrobial concentrations in polymorphonuclear leukocytes. Antimicrob. Agents Chemother. **31**:1904–1908.
10. **Nelson, R. D., R. T. McCormack, and V. D. Fiegel.** 1978. Chemotaxis of human leukocytes under agarose, p. 25–42. *In* J. I. Gallin and P. G. Quie (ed.), Leukocyte chemotaxis. Raven Press, New York.
11. **Prokesch, R. C., and W. L. Hand.** 1982. Antibiotic entry into human polymorphonuclear leukocytes. Antimicrob. Agents Chemother. **21**:373–380.
12. **Simon, H. J., and E. J. Yin.** 1970. Microbioassay of antimicrobial agents. Appl. Microbiol. **19**:573–579.
13. **Van der Auwera, P., T. Matsumoto, and M. Husson.** 1988. Intraphagocytic penetration of antibiotics. J. Antimicrob. Chemother. **22**:185–192.