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NOTES

Intracellular and Extracellular Penetration of Azithromycin into Inflammatory and Noninflammatory Blister Fluid

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The penetration of azithromycin into the blister fluids of six volunteers was analyzed after a 5-day regimen (total of 1.5 g). Differences in drug concentrations in a paper disk and serum and in the mass of azithromycin from inflammatory blister chamber leukocytes and noninflammatory blister chamber leukocytes were significant ($P < 0.05$).

Skin blisters induced by suction can be injected with autologus serum to induce a local inflammatory reaction, with leukocyte (WBC) counts as high as 10^6 to $10^9/\mu l$ after 12 to 24 h (6, 11, 12, 15, 16). The presence of leukocytes in the blisters makes them more analogous to an infectious site than suction blisters without WBCs. We anticipated that the concentration of azithromycin would be higher in inflammatory blister fluid and remain elevated for a prolonged period of time than in noninflammatory blister fluid (4, 9). This was a multiple-dose study to ascertain the penetration of azithromycin into inflammatory and noninflammatory blister fluid.

(This work was presented in part at the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 17 to 20 October 1993.)

Written informed consent and the study protocol were approved by the Hartford Hospital Investigational Review Committee. Five healthy males plus one healthy female who met all of the inclusion criteria and none of the exclusion criteria signed the approved consent forms and were admitted into the study.

Blisters (three per subject) were created approximately 12 h prior to the scheduled dose on days 1, 3, and 5. After the blisters were made, the fluid was aspirated and analyzed for protein content and WBC count. Two blisters were unroofed, and sterile, hollow chambers with a circumference slightly larger than the circumference of the denuded blister were glued with Skin-Bond (Smith and Nephew United, Inc., Largo, Fla.) to the intact skin over two of the blisters and firmly taped in position. These held either normal saline (NS) (noninflammatory) or a WBC attractant (inflammatory) over the denuded area. The third blister was left intact. The first dose of azithromycin was administered 12 h after the blisters were made. A baseline blood sample and chamber sample were obtained prior to administration of the first dose.

The third blister was sampled differently. After administration of the dose the blister was unroofed, a paper disk was placed over the blister base, 20 μl of 0.85% NaCl was spotted on the disk, and a plastic coverslip was placed over the disk. The disk was removed from the blister site after 1 h, and a new disk was placed on the site. Disks were harvested at hourly intervals beginning 2 h prior to the expected time of peak concentration in serum, at the expected time of peak concentration, for 5 h after the time of expected peak concentration, and at 12 and 24 h. Disks were stored at −70°C until assayed.

Dosing and sampling lasted for 5 days, with 500 mg of azithromycin being given on day 1 and 250 mg being administered on days 2 to 5. Blood sampling coincided with disk sampling.

Five hundred microliters of NS was injected into one chamber (noninflammatory), and 500 μl of autologus serum (inflammatory) obtained prior to day 1 was injected into the second chamber. The chambers were sampled at 12-h intervals beginning the morning of day 1, at the expected time of appropriate WBC accumulation, and again at 12 and 24 h after the time of administration of the first dose. After aspiration of the 500-μl sample, the chambers were washed twice with NS. The fluid in each chamber was replaced with either NS or autologus serum after each sampling up to 24 h after azithromycin administration.

This process was repeated on days 3 and 5 of dosing. Additional single-day samples of blister fluid and serum were collected in the morning at 48 h (day 7), 120 h (day 10), and 216 h (day 14) after administration of the last dose (day 5) from blisters which were made approximately 12 h prior to sampling on each of these days. For the single point determinations on days 7, 10, and 14, only two blisters were needed, one blister with autologus serum and one with NS.

Blood samples were centrifuged, and then the serum was removed and frozen at −70°C until assayed. Blister fluid samples were removed from the chambers and then vortexed. The volume of the fluid was recorded, an aliquot was removed for determining the WBC count and differential, and the remaining sample was centrifuged. Aliquots of the supernatant were removed for determination of azithromycin extracellular concentration and protein count determination and then frozen until assayed. The two washes from the blister chamber were combined to collect all of the WBCs, and a 100-μl aliquot was removed for WBC count and differential determination.

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The WBC pellet was washed once with 200 μl of NS, then resuspended in 0.5 ml of NS, and stored at −70°C for determination of intracellular azithromycin concentration.

Drug concentrations in serum, chamber fluid, and disks and mass within WBCs were measured by a microbiological assay with *Micrococcus luteus* ATCC 9431, similar to methods previously described (7, 8). Standard curves were then made for azithromycin in serum, saline, and a 50% phosphate buffer–50% serum solution for measuring paper disk concentrations. Paper disk standards were spotted with 26.2 μl of the solution.

Quality control (QC) samples of 0.5 and 0.05 μg/ml were made separately for the serum and saline. The high-QC sample for the paper disks was 0.75 μg/ml. Interday coefficients of variation (CVs) for serum and saline were 6.57 and 4.77% for the 0.5-μg/ml QC samples and 3.94 and 5.13% for the 0.05-μg/ml QC samples, respectively. Intraday validation yielded CVs of 2.52 and 2.25% for the high-QC samples of serum and saline and 1.96 and 2.33% for the low-QC samples, respectively. The linearity and sensitivity of the assay were 0.02 to 0.75 μg/ml.

The paper disk interday validation produced CVs of 4.44 and 4.97% for the interday high- and low-check samples, respectively, while the intraday validation CVs were 5.97 and 4.23% for the high- and low-check samples, respectively. The linearity and sensitivity of this assay were 0.02 to 3.00 μg/ml.

WBC counts from the blister chamber fluids were performed on a Coulter Stak-S model F, and differentials were interpreted with a Wright-Giemsa stain (13).

Protein counts were taken only from blister chamber samples on the morning of day 1, the evening of day 5, the morning of day 6, and the morning of day 14.

A Wilcoxon signed-rank test was used for comparison of the WBCs and differentials of the two blister chambers (significance defined as $P < 0.05$). A paired t test was used for comparing the protein counts obtained from the two different blister chamber fluids.

Comparisons were made among the following results: serum drug concentrations versus paper disk drug concentrations on days 1, 3, and 5; concentrations in extracellular inflammatory fluid and in noninflammatory fluid; and total masses of azithromycin from the WBCs in the inflammatory and the noninflammatory chambers.

An area under the curve (AUC) from 0 to 24 h (or 0 to 168 h in the case of the time period from day 7 to day 14) was calculated by using the trapezoidal rule for each subject's datum points on each day of sampling in order to incorporate the observed value, i.e., concentration and mass, etc., with respect to time. Except for the concentrations in blood and on paper disks, these AUCs consisted of three datum points; those at time zero and 12 and 24 h for days 1, 3, and 5 and single datum values combined for days 7, 10, and 14. There were no detectable serum drug concentrations on days 10 and 14.

Statistical analysis of the comparisons of the transformed data (natural log AUCs [Ln AUCs]) from the above-mentioned fluids and cells was performed by using a standard repeated measures analysis of variance under a SAS general linear model (14).

The median time to maximum concentration of the drug in serum was 2.5 h, the median maximum concentration of the drug in serum was 0.36 μg/ml, and the median AUC from 0 to 24 h was 2.37 μg · h/ml for the first dose (500 mg). These values fall within the range of reported values from single-dose (500 mg) studies (5, 7).

Median data for the WBC counts and differentials and mean data for the protein counts were as follows: the number of WBCs per cubic millimeter mm^3 for the inflammatory chamber was 12,400 (range, 1,500 to 97,500), in contrast to the value of 2,800 for the noninflammatory chamber (range, 300 to 66,000) ($P < 0.025$); the percentages of polymorphonuclear leukocytes (PMNs) were 97% (range, 86 to 100%) for the inflammatory WBCs and 95% (range, 5 to 100%) for the noninflammatory WBCs (difference not statistically significant); the protein counts were 6.6 ± 0.44 g/dl for the inflammatory fluid samples and 0.90 ± 0.31 g/dl for the noninflammatory fluid samples ($P < 0.005$).

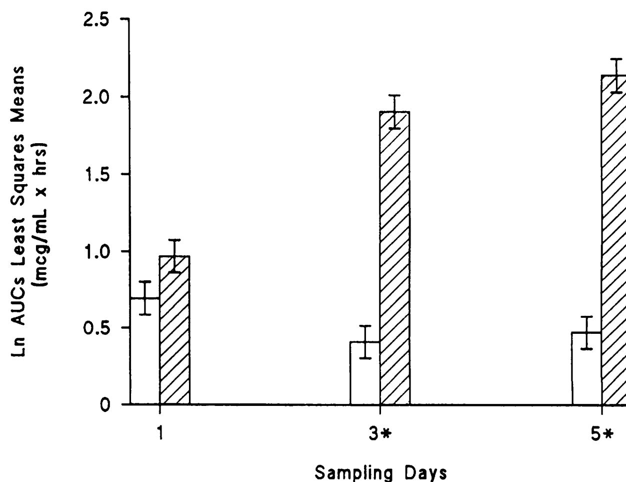

FIG. 1. Comparison of the Ln AUCs of serum (□) and paper disk (▨) azithromycin concentrations on study days 1, 3, and 5. *, $P \leq 0.001$.

The values displayed in Fig. 1 to 3 are the least-squares means of the Ln AUCs for each data set from the SAS general linear model analysis of variance. Significance was defined at a level of $P < 0.05$. One can observe a gradual increase in the concentration (Fig. 1 and 2) or mass of azithromycin (Fig. 3) over time.

Similar studies have demonstrated that azithromycin

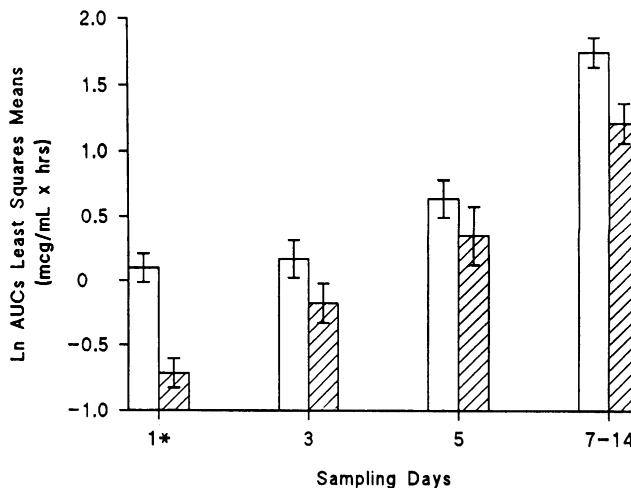

FIG. 2. Comparison of the Ln AUCs of the intracellular mass (amount) of azithromycin from WBCs obtained from inflammatory (□) and noninflammatory (▨) blister chambers on days 1, 3, 5, and 7 to 14. *, $P = 0.008$.

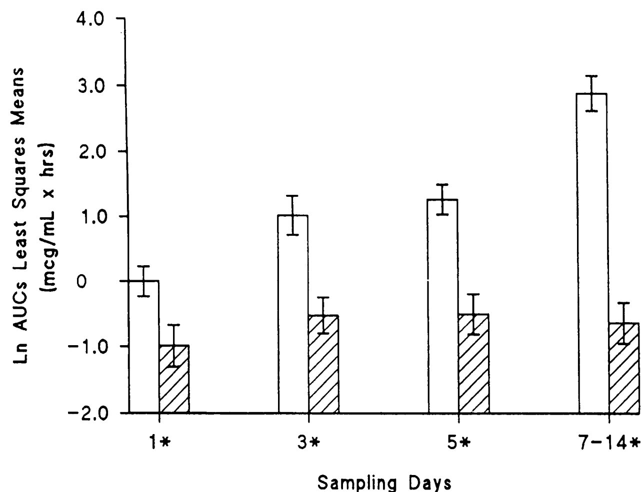

FIG. 3. Comparison of the Ln AUCs of the azithromycin concentrations in extracellular fluids obtained from inflammatory (□) and noninflammatory (▨) blister chambers on days 1, 3, 5, and 7 to 14. *, $P \leq 0.0218$.

reaches greater concentrations in extracellular fluids, particularly inflammatory fluids (2), than in serum or plasma (5). Although our results showed an increase in azithromycin concentration (Fig. 2), the extracellular inflammatory fluid only reached a statistically significant difference compared to the noninflammatory fluid on day 1. This may be due to the fact that the report by Ballow et al. did not mention separating out the WBCs from the fluids obtained from the different types of blisters (2). However, one can observe a gradual increase in concentration (Fig. 1 and 2) or mass (i.e., amount) of azithromycin (Fig. 3) over time. This is not unexpected, given azithromycin's long half-life and probable accumulation (7, 10). Gladue et al. (9), Bonnet and Van der Auwera (4), and Bermundez et al. (3) have performed in vitro studies that have found that azithromycin moves into cells such as PMNs or macrophages via active and passive transport mechanisms at a low rate. An ex vivo study which examined azithromycin concentrations in PMNs obtained from eight normal volunteers who received the same 5-day regimen of azithromycin as in this study confirmed the high intra-PMN concentrations and accumulation of azithromycin within these cells (1).

The results of this study provide additional evidence that azithromycin does indeed concentrate in, or on, PMNs and as a result may provide a unique physiological drug delivery system by which azithromycin can reach the site of an infection.

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