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Protection of Staphylococci Ingested by Macrophages from the Bactericidal Action of Rifampin

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The ability of rifampin to kill *Staphylococcus aureus* which have been ingested by normal mouse peritoreal macrophages in vitro has been investigated. In contrast to data which have been reported from experiments with other cell types, in this system rifampin was no more active than was penicillin against the two strains tested.

Rifampin exerts antimicrobial activity against a wide variety of microbes and appears to have a capacity to penetrate tissues that few other antibiotics possess. One of its theoretical advantages is its ability to penetrate intact phagocytic cells and inactivate intracellular bacteria (3). This action of the drug has been shown by several investigators who have evaluated cells as diverse as normal human polymorphonuclear leukocytes (8, 11), polymorphonuclear leukocytes from patients with chronic granulomatous disease (6), and murine peritoneal exudate cells (7). Those investigators evaluated the survival of interiorized rifampin-susceptible *Staphylococcus aureus* during in vitro incubation with various concentrations of rifampin. However, when evaluated against *Mycobacterium tuberculosis* in human monocyte cultures (4), rifamin appeared no more capable of inactivating intracellular organisms than cycloserine or ethambutol, drugs which have no notable ability to penetrate intact mammalian cells. Since there are other differences in the physiology of polymorphonuclear leukocytes, induced peritoneal exudate cells, resident peritoneal macrophages, and blood monocytes, we have evaluated the ability of rifampin to inactivate *S. aureus* 502A in mouse peritoneal macrophages which were neither induced, stimulated, nor activated. In these experiments, rifampin (2 and 10 μg/ml) was found to be no more effective than penicillin G (2 U/ml), an antibiotic which manifests little if any activity against staphylococci entrapped within phagocytic cells (1, 5, 8).

Macrophages were obtained by peritoneal lavage with phosphate-buffered saline (pH 7.4) from normal female CF-1 mice (Charles River Breeding Laboratory) and cultivated in vitro as monolayers in medium 199 and heat-inactivated newborn calf serum (TCM) plus penicillin G (100 U/ml). *S. aureus* 502A and a clinical isolate of *S. aureus* were grown as tumbling cultures overnight in Trypticase soy broth (BBL Microbiology Systems).

Potassium penicillin G was dissolved in phosphate-buffered saline, and rifampin was dissolved in dimethylsulfoxide and acidified water (9); fresh stock solutions of antibiotics were prepared weekly and stored at −20°C. Both strains of staphylococci (5×10^5/ml) were susceptible to both antibiotics when tested by tube dilution in TCM. Penicillin inhibited the growth of *S. aureus* 502A at 0.25 U/ml and caused a 99.9% decrease in viability at 2 U/ml, whereas rifampin was inhibitory at 0.05 μg/ml and bactericidal at 0.2 μg/ml. The clinical isolate was inhibited by 0.25 U of penicillin per ml and 0.025 μg of rifampin per ml.

After cultivation in vitro for 2 days, macrophage monolayers were rinsed four times with medium 199 and incubated an additional 4 h in TCM containing 1,000 U of penicillinase per ml (Difco). After an additional four rinses, the macrophages were allowed to ingest staphylococci (5×10^6 to 7×10^6 colony-forming units per ml; approximately 10 bacteria per macrophage) for 1 h. Phagocytosis was terminated by rinsing the cells with medium 199 and incubation in TCM containing lysostaphin (2 to 4 U/ml) (Schwarz/Mann) for 15 m. After lysostaphin treatment, the tubes were again rinsed four times, and fresh TCM containing either penicillin (2 U/ml) or rifampin (2 or 10 μg/ml) was added. Staphylococci were enumerated by plate counts of appropriately diluted samples of medium and of distilled water lysates of monolayers. In preliminary experiments, medium was tested for residual penicillinase or lysostaphin activity by using the medium as a diluent for a minimum inhibitory concentration assay against *S. aureus* 502A; no activity was detected.

Despite the greater in vitro activity of rifampin against the organism, in preliminary experiments there was no apparent difference in

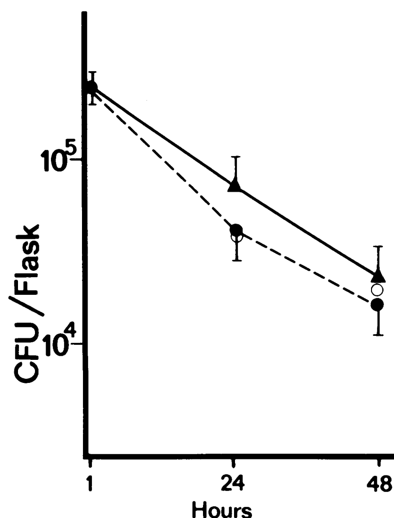

FIG. 1. *Decrease in numbers of viable cell-associated S. aureus 502A in presence of penicillin G at 2 U/ml (▲) or rifampin at 2 μg/ml (●) or at 10 μg/ml (○). Fresh medium was added at 1, 6, and 24 h. Data are plotted as means ± standard error of results from four or more experiments (▲, ●) or means of results from two experiments (○). There is no significant difference among the values at any time point.*

survival of intracellular staphylococci in macrophages in medium containing penicillin (2 U/ml) or rifampin (2 μg/ml). Consequently, because of the possibility that the rifampin might have become inactive during in vitro culture, fresh antibiotic was added at 1, 6, and 24 h after initiation of phagocytosis. Again, as is illustrated in Fig. 1, rifampin did not significantly enhance the intracellular inactivation of *S. aureus* 502A by mouse peritoneal macrophages in vitro. It was found that increasing the concentration of rifampin to 10 μg/ml, a concentration which was approximately 50 times the minimum bactericidal concentration of the antibiotic in cultures lacking macrophages, did not enhance the rate of intracellular inactivation of *S. aureus* 502A. Furthermore, log-phase organisms and stationary-phase organisms were inactivated at identical rates in macrophage cultures containing either antibiotic; these data have been combined in Fig. 1. Although the rate of kill of the organisms was slow, it was comparable to rates that we (10) and others (2) have previously described.

In two additional experiments using the clinical isolate of *S. aureus*, virtually identical results were obtained.

In summary, these data indicate that the activity of rifampin against intracellular staphylococci does not appear to be manifest when the bacteria are entrapped within noninduced mouse peritoneal macrophages. These data suggest that the apparent superiority of rifampin as an agent with the ability to kill intracellular organisms may be highly dependent on the characteristics of the ingesting phagocytic cells. Whether the cells of the fixed reticuloendothelial system, e.g., Kuppfer cells or splenic macrophages, behave like noninduced mouse peritoneal macrophages or like induced peritoneal monocytes and polymorphonuclear leukocytes cannot, of course, be determined at this time.

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