Fusidic Acid Is Highly Active against Extracellular and Intracellular Mycobacterium leprae

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The activity of fusidic acid against Mycobacterium leprae was studied in axenic medium and in bacilli residing within mouse peritoneal macrophages. Activity was assessed by subsequent quantitation of bacillary radiop-resprometric activity. Significant inhibition in both systems was observed at 0.156 μg/ml, and an approximately 50% reduction in activity occurred after exposure to 1.25 to 2.5 μg/ml. The excellent human pharmacokinetics and in vitro activity of fusidic acid against the leprosy bacillus warrant a clinical trial of this drug for leprosy.

Fusidic acid has been in clinical use in Europe for over 20 years, primarily as an antistaphylococcal agent (15). It is a naturally occurring, highly lipophilic, tetracyclic triterpenoid compound, devoid of steroidlike activity because of its stereochemistry. In humans, fusidic acid, with a 10-h serum half-life (24), is distributed well into all body compartments, with the exception of cerebrospinal fluid, and possesses very little host toxicity (21). A single 500-mg oral dose results in a maximum concentration in plasma of 33 μg/ml (24); repeated doses result in concentrations of 100 to 200 μg/ml (21). Intracellular fusidic acid concentrations are 40 to 100% extracellular concentrations (3). Its spectrum of activity is confined primarily to gram-positive bacteria, with most gram-negative bacteria being completely resistant (26). Recent reports (17, 25) noted in vitro activity against Mycobacterium tuberculosis at 16 to 64 μg/ml and, in combination with ethambutol, against some Mycobacterium avium complex strains.

In vitro screening for drugs active against the uncultivable leprosy bacillus can be performed by radiopresprometric analysis of viable Mycobacterium leprae freshly harvested from nude mice (5–7, 9–12). In vitro systems have many advantages over the traditional mouse footpad system (22), especially with regard to time, expense, quantity of drug required, and independence of mouse pharmacokinetics. Here we report on the use of radiopresprometry to examine the activity of fusidic acid in both extracellular and intracellular M. leprae.

MATERIALS AND METHODS

M. leprae. M. leprae was harvested from the footpads of infected nude mice and partially purified by differential centrifugation as previously described (11). In brief, footpads containing approximately 1010 acid-fast bacilli were surface decontaminated by 2 to 3 min of exposure to UV light, 15 min of immersion in 1% iodine (Acudyn; Acme United, Fairfield, Conn.), and repeated rinsing in absolute ethanol. Tissue was minced with scissors and homogenized in 7H12 medium (0.47 g of Middlebrook 7H9 broth [Difco Laboratories, Detroit, Mich.], 0.5 g of bovine serum albumin, 0.1 g of Casitone, 0.75 g of glucose, 400 μg of catalase, 100 ml of deionized water). Large tissue debris was removed by centrifugation (108 × g, 5 min, 10°C), and soluble tissue material was removed by pelleting of bacilli (2,710 × g, 45 min, 10°C). Bacilli were resuspended at approximately 109/ml in 7H12 medium and treated with 50 μg of ampicillin and 2.5 μg of amphotericin B per ml for 4 h to eliminate possible contaminants. These antibiotics have been shown to have no deleterious effect on M. leprae (3). The suspension was stored at 4°C overnight and used on the following day following appropriate dilution in either 7H12 medium or RPMI (GIBCO, Grand Island, N.Y.) supplemented with 20 mM glucose, 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (GIBCO), 2.5 g of NaHCO3 per liter, 2 mM glutamine, 10% dialyzed heat-inactivated fetal calf serum (HyClone Laboratories, Inc., Logan, Utah), and 50 μg of ampicillin per ml.

Fusidic acid. Sodium fusidate was obtained from Sigma Chemical Co. (St. Louis, Mo.). The drug was solubilized in either Middlebrook 7H9 broth or RPMI at 400 μg/ml and diluted in the same medium. Drug concentrations referred to are those of sodium fusidate.

Extracellular cultures. M. leprae (107) was placed in BACTEC 12B medium (Bectin Dickinson and Co., Towson, Md.) containing 50 μg of ampicillin and 2.5 μg of amphotericin B per ml. Each drug dilution was added in a volume of 200 μl in quadruplicate. Twelve cultures served as drug-free controls and received 200 μl of Middlebrook 7H9 broth. Cultures were flushed with 2.5% O2-10% CO2-87.5% N2, in a BACTEC 460 instrument and then incubated at 33°C. The growth index (14CO2 evolution) was measured at weekly intervals by flushing the cultures with the same gas mixture. The second weekly reading was used for data analysis.

Intracellular cultures. Resident peritoneal macrophages were harvested from Swiss Webster retired breeder mice (Simonsen Laboratories, Gilroy, Calif.) as described by Sibley and Krakenhul (23). Approximately 2 × 106 peritoneal cells were seeded on LUX coverslips (Miles Laboratories, Naperville, Ill.) in 24-well tissue culture plates containing RPMI. Nonadherent cells were removed by washing, and adherent cells (>90% macrophages) were infected with 107 M. leprae. After 4 h at 37°C, coverslips were removed, rinsed in Hankes balanced salt solution, and transferred to wells containing 1 ml of RPMI. Quadruplicate wells received drug dilutions in 50 μl of RPMI. Twelve wells served as drug-free controls and received 50 μl of RPMI. Plates were incubated at 33°C under 5% CO2 for 1 week. Coverslips were

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transferred to wells containing 0.4 ml of 0.1 N NaOH to lyse macrophages and release intracellular M. leprae. After 5 min at room temperature, 0.3 ml of the bacterial suspension was transferred to 4 ml of 7H12 medium adjusted to pH 5.8 (with citric acid) and containing 1 μCi of [1-14C]palmitic acid (58 mCi/mmol; New England Nuclear Corp., Boston, Mass.) per ml in 6-ml screw-cap "shorty vials" (Wheaton Scientific, Millville, N.J.). The low-pH medium obviated the need for prior neutralization of the alkaline macrophage lysate and resulted in a final medium pH of approximately 6.5. The loosely capped vials were placed within tightly capped wide-mouth scintillation vials (Poly-Q; Beckman Instruments, Brea, Calif.) containing a strip (2 by 4 cm) of Whatman no. 42 filter paper which had previously been saturated with a mixture of 20 ml of Liquiflour (New England Nuclear), 15 g of 2,5-diphenyloxazole, 5 ml of Triton X-100, and 5 ml of 2 N NaOH (in methanol) and dried overnight (2). The double-vial assemblies were incubated at 33°C, and cumulative evolved 14CO2 was measured daily for 1 week in a Beckman model LS-5801 liquid scintillation counter. The 7-day cumulative reading was used in data analysis.

Statistical analysis. Student's t test was used to test the significance of experimental results.

RESULTS

Fusidic acid effected a clear dose-dependent inhibition of metabolic activity in both extracellular (Fig. 1) and intracellular (Fig. 2) M. leprae. In both cases, significant inhibition was observed at 0.156 μg/ml (P < 0.01), with concentrations of 1.25 to 2.5 μg/ml resulting in an approximately 50% reduction in activity. At the high dose of 20 μg/ml, the activities of extracellular and intracellular bacilli were 26 and 12% those of drug-free controls, respectively. Replicate experiments (two for extracellular and one for intracellular bacilli) confirmed these results (data not shown). In addition, activity against extracellular M. leprae, as determined by the phenolic glycolipid-1 synthesis assay (16), was observed at 0.2 to 2.0 μg/ml (data not shown).

DISCUSSION

We have previously demonstrated the utility of radiorespirometry in evaluating the in vitro activities of a variety of macrolides (9), fluoroquinolones (11), phenazines (10, 12), and aminoglycosides, lincomamides, and rifamycins (7) against M. leprae in axenic media with either a Budemeyer-type detection system (5) or the BACTEC 460 instrument (6) as a means of assessing drug activity in the absence of bacterial multiplication. We and our colleagues have also used assays quantitating phenolic glycolipid-1 synthesis and adenosine triphosphate concentration (8) as indices of drug activity against both extracellular (16) and intracellular (20) M. leprae. However, the sensitivity, reproducibility, and automated nature of the radiorespirometric systems have resulted in their becoming the primary indices of M. leprae viability in our laboratory. We recently reported on the use of radiorespirometry in monitoring the effect of macrophage activation on the viability of M. leprae (1, 19). The present report is the first to use this method to study drug activity against intracellular M. leprae and to study the activity of a single drug both intracellularly and extracellularly. The roughly equal activity of fusidic acid against both intracellu-
lar and extracellular *M. leprae* is consistent with the good intracellular penetration of this drug (3).

Both the BACTEC 460 instrument and the Buddemeyer-type detection system can be used to quantitate \( ^{14} \text{CO}_2 \) evolution from L-[\( ^{14} \text{C} \)]palmitic acid with either intracellular or extracellular *M. leprae*. However, we have found the BACTEC 460 instrument to be slightly simpler to use and to produce more reproducible results, making it the system of choice for routine drug screening in axenic medium (6, 7, 11). Conversely, the Buddemeyer-type detection system is more sensitive (because of the amplification of the signal in the liquid scintillation counting system), and we often find it more useful when working with macrophage-resident (1, 19) or biopsy-derived *M. leprae*.

The in vitro activity of fusidic acid against *M. leprae* approximates or is superior to that previously found with proven agents, such as rifampin (5-7) and ofloxacin (11), and with clarithromycin (6, 9), minocycline (6), and sparfloxacin (11), drugs active against *M. leprae* in vivo and now in leprosy clinical trials. The above-listed agents effect a significant reduction in the radioespirometric activity of *M. leprae* at concentrations ranging from 0.031 to 0.5 \( \mu \text{g/ml} \). Traditional single doses of these drugs typically result in maximum concentrations in plasma of 1.5 to 10 \( \mu \text{g/ml} \), which are markedly lower than that obtained with fusidic acid (33 \( \mu \text{g/ml} \) [24]). This high ratio of maximum concentration in plasma to in vitro minimal effective concentration suggests potential clinical efficacy in leprosy. In addition, fusidic acid, which inhibits bacterial protein synthesis by binding to elongation factor G, may act synergistically with rifampin (4), a highly bactericidal antileprosy drug. Finally, the drug is also available in a topical formulation (13), offering the possibility of an additional mode of administration in leprosy.

We are unaware of any existing reports on the activity of fusidic acid against *M. leprae*. However, in marked contrast to the complete absorption that occurs upon oral dosing in humans (24), very low levels of fusidic acid in blood are obtained in mice (14), possibly precluding the detection of activity in the traditional mouse footpad model (22).

Fusidic acid is a remarkably safe, thermostable (18), orally administered antibiotic with excellent pharmacokinetics and in vitro activity against intracellular and extracellular *M. leprae*. A clinical trial of this drug for leprosy is warranted.

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