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Azithromycin, Rifabutin, and Rifapentine for Treatment and Prophylaxis of *Mycobacterium avium* Complex in Rats Treated with Cyclosporine

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Azithromycin, rifabutin, and rifapentine were used to treat or prevent disseminated *Mycobacterium avium* complex (MAC) infections produced in rats immunosuppressed with cyclosporine. Animals with bacteremic infections were treated 1 week after intravenous inoculation with 10^7 CFU of MAC with azithromycin, 100 mg/kg of body weight administered subcutaneously for 5 days and then 75 mg/kg on Monday, Wednesday, and Friday, or with rifabutin or rifapentine, 20 mg/kg administered intraperitoneally on Monday through Friday. All three drugs showed efficacy after 1 and 2 months. Rifabutin cleared the organisms from tissues more rapidly than azithromycin or rifapentine. To approximate prophylaxis, treatment was started 2 weeks before intravenous inoculation with 10^4 organisms. MAC infections were undetectable in treated animals after 4 months, while control animals had disseminated infections. These findings support the rationale for clinical trials of treatment and prophylaxis with these agents. The cyclosporine-treated rat appears to be a useful model in which to evaluate compounds for the treatment and prophylaxis of disseminated MAC infections.

Disseminated infection with the *Mycobacterium avium* complex (MAC) is the most common cause of bacteremia in patients with AIDS, occurring in up to 50% of these patients (14). Survival of MAC- and human immunodeficiency virus-infected patients is half that of those with similar stages of human immunodeficiency virus infection but who do not have disseminated MAC infections (13). Treatment regimens with significant activity against MAC have been described, but relapses have occurred and none of the regimens have reliably eradicated the infection (9, 10, 11, 20, 25). Thus, there is an urgent need for more effective agents and approaches to the treatment and prevention of MAC infections.

Successful treatment of established infections probably requires combination therapy with two or more effective drugs having different mechanisms of action (17). Rifabutin and rifapentine are new rifamycin analogs which demonstrate significant in vitro activity against MAC isolates. Studies of treatment with these agents in animal models have produced contradictory results (4, 5, 15, 16). Azithromycin is a new azilide antibiotic which, despite its low in vitro activity against MAC, has shown promise as a treatment in animal studies (12) and early human trials (26). This efficacy may stem from its unusual pharmacokinetic profile, which can result in high concentrations in tissues (6).

A new model of disseminated MAC infection has recently been described in Sprague-Dawley rats immunosuppressed with cyclosporine (CsA) (2). We evaluated the response to treatment with azithromycin, rifabutin, or rifapentine in CsA-treated rats with bacteremic MAC infections. As a model of prophylaxis, we also tested whether these drugs prevent the progression of low-grade infection.

(Results of the studies described here were presented in part at the Sixth International Symposium on Infections in the Immunocompromised Host, Peebles, Scotland, June 1990 [1a].)

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MATERIALS AND METHODS

Drugs. Azithromycin was provided by Pfizer Central Research, Groton, Conn. Rifabutin was provided by Adria Laboratories, Dublin, Ohio. Rifapentine was provided by Merrell-Dow Pharmaceuticals, Cincinnati, Ohio. Stock solutions of azithromycin were prepared in buffered saline (pH 6.8). Rifabutin was dissolved in 95% ethanol, and rifapentine was dissolved in 100% dimethylformamide. On the morning of administration, stock solutions of azithromycin were diluted with 10% polyoxyethylated castor oil (Cremophor El; BASF, Ludwigshafen, Germany) in saline, while rifabutin and rifapentine were diluted with saline only. The final concentration of ethanol or dimethylformamide for the rifamycins was ≤0.5%. The dose and schedule of drug administration were selected to achieve therapeutic levels similar to those obtained in humans on the basis of the available pharmacokinetic data (6, 15).

Mycobacterial strain. Strain SK095, previously designated strain SK005 (2), was isolated from the synovial fluid of a patient with AIDS and disseminated MAC at the Memorial Sloan-Kettering Cancer Center. It was identified as *M. avium* by nucleic acid hybridization (GEN-PROBE, San Diego, Calif.) and belonged to restriction fragment length polymorphism group A, as determined by the method of McFadden et al. (19). No serotype could be determined by methods used at the National Jewish Center for Immunology and Respiratory Medicine, Denver, Colo., or the Centers for Disease Control, Atlanta, Ga. (3, 7).

After isolation and second passage on 7H11 agar, predominantly flat, transparent isolates were frozen at −70°C as aliquots of 10^8 CFU/ml in 7H9 broth until use in the studies described here.

TABLE 1. Drug susceptibility of *M. avium* SK095[a]

Drug	MIC (μg/ml) at:	
	pH 6.5	pH 7.5
Azithromycin	128	32
Rifabutin	0.125	0.125
Rifapentine	0.25	0.25

[a] Susceptibilities were determined by the broth microdilution method.

The susceptibilities of SK095 to the study drugs, shown in Table 1, were determined by a previously described broth microdilution method (21).

CsA-treated rats. The model of disseminated infection with MAC in rats treated with CsA was used (2). Male Sprague-Dawley rats (weight, 150 to 175 g; Charles River Laboratories, Wilmington, Mass.) were fed standard rat chow and water ad libitum. Animals were immunosuppressed with CsA (Sandimmune; gift from Sandoz Corp., Nutley, N.J.) that was dissolved in polyoxyethylated castor oil, diluted with saline, and injected subcutaneously in 0.3 to 1.0 ml at 20 mg/kg of body weight on Monday and Wednesday and at 40 mg/kg on Friday. No prophylactic antimicrobial agents were used. Anesthesia with ethrane (Enflurane; Anaquest, Memphis, Tenn.) was used during all noxious procedures and prior to sacrifice by asphyxiation with CO_2. The protocols for studies in animals were approved by the Institutional Animal Care and Use Committee of the Memorial Sloan-Kettering Cancer Center.

Infectious inocula. Thawed aliquots of strain SK095 were cultured on 7H11 agar plates enriched with oleic acid, albumin, glucose, and catalase (BBL, Bethesda, Md.) and were incubated for 3 to 5 days at 37°C. Organisms were harvested in sterile water containing 0.05% Tween 80, and single-cell suspensions in saline were prepared by low-speed centrifugation. The concentration of organisms in the supernatant was determined by measuring the optical density, and dilutions were made in saline. Concentrations of viable organisms were determined by counting colonies from serial 10-fold dilutions on 7H11 agar plates.

Assays. The extent of infection was determined from serial quantitative cultures of blood, spleen, liver, and lung. Blood was obtained by cardiocentesis prior to sacrifice and was incubated in 1.5-ml VACUTAINER tubes containing saponin (Pediatric Isolater tubes; DuPont Co., Wilmington, Del.) to release intracellular organisms. Weighed specimens of organs were homogenized at a dilution of 1:10 (by weight) in 0.1% sodium deoxycholate in saline (24). Serial dilutions of homogenates or blood were incubated on 7H11 plates at 37°C, and the colonies were counted after 7 and 14 days.

Trough levels of drugs in tissues were determined from samples that were taken when the animals were sacrificed on Monday mornings, approximately 3 days after the last dose of study drug. Concentrations of azithromycin and rifapentine in tissue were determined by bioassay with *Micrococcus luteus* ATCC 10240 by a standard pour plate technique (6). The zones of inhibition produced by serial dilutions of homogenates of serum and tissue from treated animals were compared with the zones produced by measured concentrations of drug diluted in the corresponding tissue from untreated controls. Rifabutin concentrations were measured by a high-performance liquid chromatography assay modified from previously described methods (1).

Serum CsA levels were measured with one of two commercial radioimmunoassay kits (one was a gift from Sandoz Corp. and the other one was obtained from INCSTAR Corp., Stillwater, Minn.).

Statistics. Results of quantitative cultures are expressed as the mean ± 95% confidence limits from sample sizes of five animals per datum point unless indicated otherwise. Analysis of variance was performed by using a computerized statistical analysis program (Statgraphics; STSC, Inc., Rockville, Md.).

RESULTS

Treatment of established infections. To study the effects of the study drugs on established infections, animals were intravenously inoculated with 10^7 CFU of MAC 10 days after CsA was started. Treatment was begun 1 week after animals were infected. Azithromycin was given subcutaneously, 100 mg/kg for 5 days and then 75 mg/kg subcutaneously on Mondays, Wednesdays, and Fridays. Rifabutin and rifapentine were given intraperitoneally at 20 mg/kg on Monday through Friday. Infection was assayed after 8 days from the start of treatment and again after 32 and 60 days from the start of treatment.

No bacteremia was detected in treated animals after 40 or 68 days of infection. Bacteremia was present in all control animals after 8, 40, and 68 days of infection. Mean counts ranged from 40 to 100 CFU/ml of blood, and there were no significant differences in the degree of bacteremia between groups of control animals during the course of the study.

Significantly fewer mycobacteria ($P < 0.01$) were recovered from the spleens and livers of treated animals after 32 days and from the lungs after 60 days when compared with the number recovered from untreated controls (Fig. 1). Two hundred-fold and greater reductions from the baseline mycobacterial concentrations ($P < 0.01$) were seen in spleens and livers after 32 days of therapy in all treatment groups and in lungs in rifabutin-treated animals. Counts from the lungs of animals treated with azithromycin and rifapentine were not significantly lower than those at the start of therapy.

Rifabutin cleared MAC from all organs more rapidly than azithromycin or rifapentine did. Mean counts from the lungs and livers were below the limits of the assay after 32 days of treatment with rifabutin. After 60 days of treatment, mean counts from the spleens of animals treated with rifabutin were 100-fold less than those from the spleens of animals treated with azithromycin ($P < 0.01$) and 200-fold less than those from the spleens of animals treated with rifapentine ($P < 0.01$).

Prophylaxis. To determine the abilities of the study drugs to prevent the progression of low-grade infection, treatment was begun 1 week after starting CsA. Treatment with azithromycin was started at a dose of 100 mg/kg on Mondays, Wednesdays, and Fridays and was changed to 100 mg/kg on Mondays and Fridays. Rifabutin and rifapentine were given at 20 mg/kg on Mondays, Wednesdays, and Fridays until the time of sacrifice. Animals were infected with 10^4 CFU of MAC 2 weeks after antimycobacterial treatment was begun. After 2 months, animals treated with azithromycin were seen to have coarse coats of hair, which suggested that they had developed a toxic response to the drug. The dosing schedule for azithromycin-treated animals was therefore changed after 80 days of treatment to 100 mg/kg on Mondays and Fridays. Three animals were randomly selected from the untreated control group and were sacrificed at 1 and 56 days after infection. All remaining animals were sacrificed after 112 days of infection.

Progressive infection in control animals was seen over the

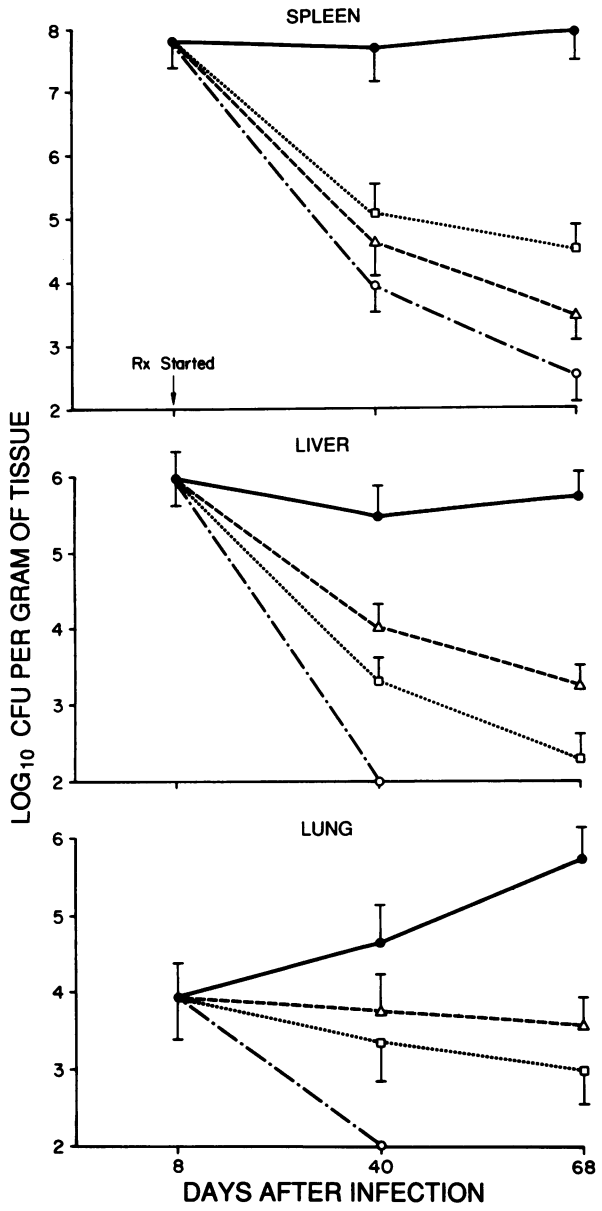

FIG. 1. Effect of treatment on degree of MAC infection over time. All animals were immunosuppressed with CsA and were infected with 10^7 CFU of MAC. Animals were treated 8 days after infection with azithromycin (△), rifabutin (○), or rifapentine (□), or were used as untreated controls (●). Datum points represent mean counts from the tissues of five animals ± 95% confidence limits.

course of the trial. After 4 months of infection, no evidence of disseminated infection was found in animals treated with azithromycin, rifabutin, or rifapentine (Table 2). Untreated animals had disseminated infections with up to 200-fold greater CFU per gram of spleen than the count in the original inoculum. Bacteremia was present in four of six control animals after 4 months but was absent in treated animals.

Drug levels. Levels of the antimycobacterial drugs were measured in the tissues of animals sacrificed after 60 days of treatment for established infection and 3 days after the latest administration of test compounds. The concentrations in serum and organ homogenates of three animals in each treatment group are given in Table 3.

TABLE 2. Effect of prophylactic treatment on MAC infection after 105 days of therapy

Treatment[a]	Log_{10} CFU/g of MAC in the following tissues[b]:		
	Spleen	Liver	Lung
Control	5.6 ± 0.5	2.9 ± 0.4	2.8 ± 0.9
Azithromycin	<2	<2	<2
Rifabutin	<2	<2	<2
Rifapentine	<2	<2	<2

[a] Animals were intravenously inoculated with 10^4 CFU MAC 2 weeks after starting antimycobacterial treatment.
[b] Mean ± 95% confidence limits from six animals per datum point.

Trough CsA levels were measured after 32 and 60 days of treatment. The combined mean serum CsA level after 32 and 60 days of treatment was 195 ± 90 ng/ml (standard deviation; range, 112 to 293 ng/ml). There were no significant differences in CsA levels within or between treatment groups over this period of time.

DISCUSSION

This is the first report of the treatment of disseminated MAC infection in CsA-treated rats. The results show that azithromycin, rifabutin, and rifapentine are effective in the treatment of bacteremic MAC infections in this model. Each of these drugs also prevented the progression of low-grade infection over 4 months.

Rifabutin was the most active of the three drugs in clearing established MAC infection from tissues. Previous reports have given conflicting accounts of the activity of rifabutin for the in vivo treatment of MAC. Furney et al. (4) have suggested that discrepancies in the activity of rifabutin observed between studies in beige mice (5) and thymectomized CD4 T-cell-deficient C57BL/6J mice (4) may be attributable to differences in the susceptibilities of the test strains used to the study drugs. Our results support this view since the strain used, SK095, was quite susceptible in vitro to rifabutin.

A similar controversy concerning the activity of rifapentine also exists (15, 16). Our findings lend support to the view that rifapentine may have significant activity against MAC infections. Most reports of in vivo therapeutic trials have evaluated therapy after treatment for 30 days or less. Our results showed that while the activity of rifapentine was only suggestive after 30 days of treatment, it was clearly signifi-

TABLE 3. Trough concentrations of drugs in tissue after 60 days of treatment for established *M. avium* infection[a]

Tissue	Mean ± SD concn (μg/g or μg/ml)		
	Azithromycin	Rifabutin	Rifapentine
Serum	0.75 ± 0.5	0.02 ± 0.01	4.7 ± 1
Spleen	895 ± 180	0.75 ± 0.53	8 ± 1
Liver	207 ± 109	0.75 ± 0.13	28 ± 9
Lung	440 ± 156	0.37 ± 0.02	11 ± 5

[a] Tissue samples from three animals in each group were assayed 60 days after the start of treatment and 3 days after the last dose was given. Levels of azithromycin in tissues were determined by a standard bioassay with *M. luteus*. Rifabutin levels were assayed by high-performance liquid chromatography.

cant after 60 days. Nonetheless, rifapentine was less active than rifabutin, despite good levels in tissues and the similar susceptibilities of the test strain to both of these compounds. The cause of these discrepancies is unknown, but differences in the tissue pharmacokinetics of these agents, such as intracellular compartmentalization or protein binding, could affect their bioavailability to intracellular MAC. Alternatively, intracellular organisms may have phenotypic characteristics that alter the in vivo susceptibility of a strain when compared with its in vitro phenotype.

Azithromycin has shown in vivo activity against MAC in animals and humans, despite poor in vitro susceptibilities. As with other macrolides, the MIC of azithromycin decreases as the pH increases (22). The MIC for MAC strain SK095 was 10 times greater than the measured concentration in serum at pH 7.5 and 60 times greater than the measured concentration in serum at pH 6.5. In contrast, the concentrations of azithromycin in tissues ranged from 10 to 100 times the MICs for the organism. These data support the concept that the antimycobacterial activity of azithromycin results from the unusually high concentrations in tissues which can be obtained with this drug. The use of in vitro susceptibilities as a guide to therapy may not be useful with this class of agents or with other compounds with long half-lives in tissues. Thus, the use of animal models remains of critical importance for the evaluation of novel treatments for MAC infections.

Pharmacokinetic interactions have been reported between CsA and other rifamycin and macrolide drugs. Since rifamycins may induce increased hepatic metabolism of CsA (23), a possible explanation for the observed therapeutic effects of rifabutin and rifapentine could be recovery of immune function because of subtherapeutic CsA levels. We found no difference in Csa levels between control animals and animals treated with the study drugs. Unidentified immunoadjuvant effects also seem unlikely because no significant granulomatous response in the tissues of treated animals was seen. This is in contrast to what is seen in animals recovering from MAC infections after withdrawal of CsA, in which a vigorous granulomatous response occurs. Macrolides may also affect the in vivo concentrations of CsA (18). The azithromycin levels obtained were within the range that would be predicted by the pharmacokinetics observed in animals not treated with CsA (6). Thus, we found no significant interaction between CsA and the study drugs.

The magnitude and urgency of the problem of MAC infection in patients with AIDS and the lack of proven therapy for established disease make consideration of prophylactic treatment strategies an attractive alternative to therapy for the acute phase of infection. The value of prophylaxis for other opportunistic infections in immunocompromised patients is well established. In the case of *Mycobacterium tuberculosis*, prophylaxis is actually treatment of a subclinical infection with a low bacillary burden, a situation analogous to the model of prophylaxis used in the studies described here. Recently conducted clinical trials show that rifabutin is effective for prophylaxis of MAC infections in humans (8), as it was in the animal model described here. These results appear to validate the CsA-treated rat as a model for studies of prophylaxis of MAC infections. The encouraging results obtained with azithromycin and rifapentine suggest that these drugs may also be useful in the prophylactic treatment of MAC infections.

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