

Experimental Observations on the Antimycobacterial Activity of Rifampin* (33868)

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In a recent communication from this laboratory (1), data were presented which confirmed the observations of Pallanza and his associates (2) and called attention to the remarkable *in vitro* and *in vivo* antimycobacterial activity of rifampin, a new semisynthetic antimicrobial agent. The present report concerns subsequent studies conducted *in vitro* to evaluate the effect of culture medium on the degree of activity of the drug and *in vivo* to evaluate its protective activity when administered to experimentally infected tuberculous mice, either singly or in combination with one or more other antituberculosis drugs.

Methods. The methods employed in all *in vitro* experiments have been described in detail elsewhere (1). In those experiments conducted *in vivo*, mice were challenged intravenously with approximately 1×10^6 microbial cell units of the JW3371 clone isolate¹ from the H37Rv strain of *M. tuberculosis*. Rifampin², ethambutol, streptomycin, and isoniazid, each used singly, were compared

with (a) rifampin administered in combination with ethambutol, streptomycin, or isoniazid, and (b) ethambutol administered in combination with streptomycin or isoniazid. Further, each of these one- or two-drug regimens was compared with a regimen containing streptomycin, isoniazid, and para-aminosalicylic acid, and each was compared also with a similar regimen in which para-aminosalicylic acid was replaced by rifampin. The drug dosages employed were 0.2 mg of rifampin, 0.3 mg of ethambutol, 0.1 mg of isoniazid, 2.0 mg of streptomycin and 4.0 mg of para-aminosalicylic acid, administered once daily 5 days/week, for a 3-week period beginning 72 hr after challenge. Drugs used in combination were administered simultaneously, with the exception of streptomycin which was given subcutaneously within 1 hr after oral administration of the other drug or drugs. All animals were observed for a period of 42 days, or until death, and were autopsied at the time of death or sacrifice; in all instances the infecting organisms were recovered at autopsy and tested for their susceptibility to the drugs employed.

Results. *In vitro observations.* The susceptibility of 20 strains of *M. tuberculosis* to rifampin was compared to their susceptibility to isoniazid, using initially a liquid Tween-albumin medium. All were susceptible to 0.006 to 0.05 (mean 0.018) μg of rifampin/ml of medium. The range of susceptibility to isoniazid was from ≤ 0.04 to > 10 (mean > 2.18) μg /ml of medium. Six of the 20 strains were resistant to at least 5 μg of isoniazid/ml; one only of the 20 was inhibited by < 0.05 μg of isoniazid, the largest amount of rifampin required to inhibit any of the strains. When Tween 80 was eliminated from the medium, it was apparent that, as was reported previously with a smaller number of strains (1), the susceptibility to rifam-

* Supported in part by the Infectious Disease Research Institute, Newark, New Jersey, through research grants from the New York Tuberculosis & Health Association, Inc., the New Jersey Tuberculosis & Health Association, Inc., the Brookline Tuberculosis & Health Society, Inc., and Chas. Pfizer & Co., Inc., New York, New York, and through Public Health Service Research Grant No. AI-05662 from the National Institute of Allergy and Infectious Disease.

¹ We are indebted to Dr. Gerard Wijsmuller, Bureau of Disease Prevention and Environmental Control, U. S. Public Health Service, Washington, D. C., for this substrain which was isolated by him from the H37Rv strain by selection of morphologically identifiable clones.

² Rifampin Lot No. P/414 was received through the courtesy of Dr. Robert Nolan, Pittman-Moore, Division of Dow Chemical Company, Indianapolis, Indiana.

TABLE I. The *in Vitro* Activity of Rifampin and Isoniazid in a Liquid Medium.

Strain of <i>M. tuber- culosis</i>	Minimal inhibitory conc ($\mu\text{g/ml}$)			
	Rifampin		Isoniazid	
	Tween- albumin	Albu- min	Tween- albumin	Albu- min
PR-146 ^a	0.006	0.20	0.08	
PR-410	0.013	0.20	5.00	>5.00
PR-441	0.025	0.20	0.16	
PR-503	0.006	0.05	5.00	
PR-123	0.025	0.20	0.16	
PR-131	0.025	0.20	0.63	
PR-147	0.050	0.40	0.08	
PR-589	0.025	0.20	>10.	>10.
PR-629	0.006	0.10	0.31	0.63
PR-841	0.013	0.20	0.31	
PR-1300	0.025	0.20	0.08	0.04
PR-1336	0.025	0.20	0.08	
PR-1346	0.013	0.05	>10.	>10.
PR-819	0.013	0.20	>10.	>10.
PR-1233	0.006	0.05	\leq 0.04	
PR-1282	0.013	0.20	0.31	
PR-1359	0.006	0.10	10.	>10.
PR-1370	0.006	0.10	0.63	
PR-1499	0.013	0.10	0.63	
H37Rv	0.050	0.20	0.08	
Mean	0.018	0.168	>2.18	

^a All PR strains are wild strains of *M. tuberculosis* isolated from patients with newly diagnosed, previously untreated tuberculosis.

pin is far greater in the presence than in the absence of Tween. That this may not be due entirely to the antimycobacterial activity of Tween is suggested by the fact that the increased effect with rifampin is greater than with isoniazid (Table I). On 7H10 agar, a much higher concentration of rifampin, *i.e.*, 0.05–1.0 (mean 0.57) $\mu\text{g/ml}$ of medium, was required for inhibition of the growth of these 20 strains. But even on this medium, isoniazid-resistant strains were highly susceptible to rifampin.

A comparison of the susceptibility of 11 of these 20 strains, and 4 others in addition, to rifampin, isoniazid, and ethambutol indicated that all were considerably more susceptible to rifampin than to either ethambutol or isoniazid (Table II).

Three of four strains of *M. kansasii* and

one of six strains of Group III mycobacteria were susceptible to rifampin, although none was susceptible to isoniazid. Those rapidly-growing strains tested were as resistant to rifampin as to isoniazid (Table III).

In vivo observations. In subsequent studies, an attempt was made to evaluate the activity of rifampin when administered singly or in combination with one or more other antituberculosis drugs. The regimens employed are shown in Table IV. The mean survival time of animals treated on all regimens except those consisting of rifampin or ethambutol alone ranged from 40.2 to 42.0 days when evaluated at 42 days after challenge. The mean survival time of animals treated with rifampin or ethambutol alone was 34.1 and 31.6 days, respectively. Untreated animals showed a mean survival time of 26.1 days when evaluated at 42 days after challenge. *M. tuberculosis* was recovered from the lungs and spleens of all animals at the time of death or sacrifice; all isolates were susceptible to 0.2 μg or less of isoniazid, 2.5 μg or less of streptomycin, 5.0 μg or less

TABLE II. The *in Vitro* Activity of Rifampin, Ethambutol and Isoniazid (7H10 agar medium with OADC enrichment).

Strain of <i>M. tuberculosis</i>	Minimal inhibitory conc ($\mu\text{g/ml}$)		
	Rifampin	Ethambutol	Isoniazid
PR-118 ^a	0.05	10.0	5.0
PR-146	0.05	10.0	\leq 0.2
PR-326	0.02	5.0	\leq 0.2
PR-410	0.05	5.0	5.0
PR-441	0.10	5.0	\leq 0.2
PR-492	0.05	5.0	1.0
PR-503	0.05	5.0	1.0
PR-581	0.05	5.0	5.0
PR-819	0.05	10.0	>10.0
PR-1233	0.05	10.0	\leq 0.2
PR-1282	0.20	5.0	\leq 0.2
PR-1359	0.10	5.0	>10.0
PR-1370	0.05	10.0	\leq 0.2
PR-1499	0.05	5.0	1.0
H37Rv	0.05	5.0	\leq 0.2
Mean	0.06	6.56	

^a All PR strains are wild strains of *M. tuberculosis* isolated from patients with newly diagnosed, previously untreated tuberculosis.

TABLE III. The *in Vitro* Activity of Rifampin and Isoniazid against Mycobacteria other than Tubercle Bacilli (7H10 agar medium with OADC enrichment added).

Mycobacterial strain ^a	Minimal inhibitory conc (μg/ml)	
	Rifampin	Isoniazid
<i>M. kansasii</i> ^b		
P1	0.1	1.0
P4	0.1	1.0
P24	1.0	>5.0
V132	0.1	1.0
Group III ^b		
V34	0.05	5.0
V37	5.0	>5.0
P39	5.0	5.0
P40	5.0	>5.0
P45	5.0	>5.0
P54	5.0	5.0
<i>M. phlei</i>	1.0	>5.0
<i>M. fortuitum</i>		
Penso-NYH60	>5.0	>5.0
No. 226	>5.0	>5.0
No. 335B	>5.0	>5.0
Kirchberg (avian)	>5.0	>5.0

^a Strains P1, P4, P24, P39, no. 226, no. 335B were received through the courtesy of Dr. Ernest Runyon, Salt Lake City (Utah) Veterans Administration Hospital. Strains V-132, V34, V-37, P40, P45, P54 were received from Dr. Daniel Jenkins, Baylor University Medical School, Houston, Texas. All other strains were received from Dr. Walsh McDermott, Cornell University Medical College.

^b Data on strains of *M. kansasii* and on Group III and avian mycobacteria represent readings made after 21 days' incubation. Data on strains of *M. phlei* and *M. fortuitum* represent readings made after 7 days' incubation at 37°.

of ethambutol, and 0.05 μg or less of rifampin.

Discussion. The data presented herein clearly establish that rifampin is a highly active antimicrobial agent, effective *in vitro* against many strains of *M. tuberculosis* and against some strains at least of *M. kansasii*. It is more effective against organisms growing in liquid than on solid media, and apparently more effective in the presence than in the absence of Tween.

Of interest is the fact that rifampin is not only active against isoniazid-resistant strains

of *M. tuberculosis* but at least as active as isoniazid against isoniazid-susceptible strains. Yet *in vivo*, in the experimental system employed in the present study, rifampin administered in a dosage equivalent to approximately 16 mg/kg per day was less effective than isoniazid in a dosage equivalent to approximately 8 mg/kg per day. That this is not due to the emergence of rifampin-resistant mycobacterial cells during the course of therapy is indicated by our failure to recover drug-resistant organisms from any of the 240 animals employed in the study. More probably, therefore, the difference is due to differences in the absorption, distribution, and excretion of these two drugs, at least in mice.

Although not demonstrated in the present study, there is no doubt, however, that mycobacterial cells resistant to rifampin will at

TABLE IV. The *in Vitro* Activity of Rifampin Alone and in Combination with Other Antituberculosis Drugs against Experimental Tuberculous Infections in Mice.

Drug regimens ^a	No. of survivors/ total no.	Mean survival time (days) ^b
RMP + EMB	14/16	40.2
RMP + SM	19/20	41.0
RMP + INH	19/20	41.0
RMP alone	9/19	34.1
EMB alone	7/18	31.6
SM alone	18/19	41.4
INH alone	19/19	42.0
EMB + SM	18/19	40.5
EMB + INH	20/20	42.0
SM + INH + RMP	19/19	42.0
SM + INH + PAS	19/20	40.8
Controls (no therapy)	5/19	26.1

^a Rifampin (RMP) 0.2 mg/mouse per day; ethambutol (EMB) 0.3 mg/mouse per day; streptomycin (SM) 2.0 mg/mouse per day; Isoniazid (INH) 0.1 mg/mouse per day; para-aminosalicylic acid (PAS) 4.0 mg/mouse per day. Streptomycin was administered subcutaneously; all other drugs were administered orally. All animals were infected intravenously with *M. tuberculosis* and treated once daily, 5 days/week for 3 weeks beginning 72 hr after challenge.

^b Calculated at 42 days after challenge.

times emerge during the course of therapy. This is clearly apparent from observations on the rate of emergence of drug-resistant cells within strains of *M. tuberculosis*, reported elsewhere by Hobby *et al.* (3, 4), and from observations on *E. coli* and *Staphylococcus aureus* recently reported by Kunin *et al.* (5). Further, it is apparent from the data presented by Grumbach and Rist (6) who observed a frequent emergence of drug-resistant cells during the course of long-term treatment of experimentally infected tuberculous mice.

In the treatment of tuberculosis in humans, the emergence of drug resistance has occurred frequently with other antimicrobial agents, but generally has been controllable by the use of appropriate combinations of drugs. The data presented herein suggest that rifampin too may be used effectively *in vivo* in combination with streptomycin, isoniazid, or ethambutol and perhaps may be used effectively in place of para-aminosalicylic acid in multiple drug regimens. Too few dosage regimens have been used to indicate if antagonism can be demonstrated. The dosages employed in man however are generally in excess; antagonism has been observed only rarely *in vivo* and generally has occurred only when one drug or the other has been employed in minimal amounts.

Summary. Experimental studies *in vitro* and *in vivo* indicated that rifampin is highly active against both isoniazid-susceptible and isoniazid-resistant strains of *M. tuberculosis*. Although less active *in vivo* than isoniazid, at least in the dosages employed, it was highly active in combination with streptomycin, isoniazid, or ethambutol, or in combination with both streptomycin and isoniazid, against experimental tuberculosis in mice produced by intravenous challenge with the H37Rv strain of *M. tuberculosis*. Emergence of microbial resistance to rifampin was not observed during a 3-week course of treatment nor during a 3-week followup period after cessation of therapy. Antagonism, moreover, between rifampin and streptomycin, isoniazid, or ethambutol was not observed.

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Received Jan. 13, 1969. P.S.E.B.M., 1969, Vol. 131