Evidence that Antituberculosis Drugs Are Really Effective in the Treatment of Pulmonary Infection Caused by *Mycobacterium avium* Complex[1,2]

MICHIO TSUKAMURA

SUMMARY Successful chemotherapy of pulmonary disease caused by *Mycobacterium avium* complex by antituberculosis drugs has been reported by a number of investigators. However, no certain evidence of the efficacy has yet been demonstrated in a controlled clinical trial. The present study has approached this problem in 2 ways: serial analysis of minimal inhibitory concentrations (MIC) during treatment and correlation of response to therapy with initial MIC. It was observed that after administration of antituberculosis drugs (rifampin, isoniazid, kanamycin, enviomycin, and minocycline), MIC values for the *M. avium* complex strain increased significantly. This change may be considered a result of suppression of relatively susceptible bacteria and as evidence of the efficacy of drugs. Furthermore, a correlation between the MIC values determined before chemotherapy with the conversion of sputum to negative was shown. The *M. avium* complex strains varied markedly in their susceptibility to antituberculosis drugs, and these susceptibilities were correlated with the chemotherapeutic effect. The fate of patients seemed to be greatly influenced by the susceptibilities of the strains that caused infection. AM REV RESPIR DIS 1988; 137:144-148

Introduction

Successful chemotherapy of patients with lung infections caused by the *Mycobacterium avium* complex using antituberculosis drugs has been reported by a number of investigators (1-9). However, a controlled study has not yet been carried out, and, therefore, the efficacy of antituberculosis drugs has not been demonstrated definitively. It is still questionable whether the improvement in patients has been caused by the efficacy of drugs or by an improvement in the host-parasite relationship. The present study was designed to approach the problem of the efficacy of antituberculosis drugs in 2 ways. After administration of a potential drug, an emergence of a resistant population should occur because of suppression of susceptible bacteria. Therefore, an increase in the minimal inhibitory concentration (MIC) after chemotherapy is evidence of the efficacy, even when the chemotherapy has failed to cause negative conversion of sputum culture (10). Second, if the chemotherapy is effective, a correlation between the drug susceptibilities and the bacteriologic response should be observed (11). These 2 possibilities have been examined in this study.

Methods

Strains

Unless specially noted, the strains used were isolated from patients who had not been treated with any antituberculosis drug. The strains were identified as members of the *M. avium* complex by methods previously described (12). The strains were maintained by lyophilization.

Susceptibility Testing

The strains were cultivated in Ogawa egg medium (12) at 37° C for 2 wk. Growing colonies were homogenized by shaking with glass beads for 10 min and suspended in a 0.1% (vol/vol) Tween® 80 solution to a concentration of 5 mg wet weight per ml. Each 0.02-ml sample of the suspension was inoculated onto Ogawa egg medium containing the antituberculosis drug or no drug by a spiral loop that can deliver a 0.02-ml sample in one inoculation. The tubes inoculated were stoppered by a gum cap with a 3-mm cut in the bottom and incubated at 37° C for 14 days (some dysgonic strains for 21 days). The MIC was determined as a concentration of the drug in which no membranous growth could occur.

The medium was poured in 7-ml aliquots into tubes 165 by 16.5 mm and made as slopes by sterilization at 90° C for 60 min. Antituberculosis drugs were added to the medium before sterilization. One volume of an agent-solution was added to 100 volumes of the medium. Rifampin (Lepetit, Milano, Italy), ethionamide (Shionogi, Osaka, Japan), and sulfadimethoxine (Chugai, Tokyo, Japan) were dissolved in propylene glycol, and streptomycin sulfate (Meiji Co., Tokyo, Japan), kanamycin sulfate (Meiji), enviomycin sulfate, a derivative of viomycin that is 2- to 4-fold more active than viomycin (Toyo Zojo Co., Shizuoka, Japan), ethambutol (Kaken Co., Tokyo, Japan), isoniazid (Shionogi), minocycline (Lederle Japan, Tokyo), and kitasamycin, an analogue of erythromycin that is less toxic (Tozo Jozo Co., Shizuoka, Japan) were dissolved in distilled water. Minocycline, sulfadimethoxine, and kitasamycin were used previously in the treatment of *M. avium* complex disease (13-15).

The test concentrations were as follows: kanamycin and enviomycin, 400, 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.6, and 0 μg/ml; rifampin, streptomycin, ethionamide, and isoniazid, 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.6, 0.8, and 0 μg/ml; ethambutol, sulfadimethoxine, kitasamycin, and minocycline, 50, 25, 12.5, 6.25, 3.13, 1.6, 0.8, 0.4, 0.2, and 0 μg/ml (a total of 100 tubes for 1 strain). As shown below, the activities of the drugs were decreased by the absorption to the protein. However, the concentrations incorporated into the medium were shown in data without modification.

Absorption of Antituberculosis Drugs to Coagulated Protein

In an aid to measure the absorption of antituberculosis drugs to the medium, the concentration of antituberculosis drugs contained in coagulation water produced by sterilization of the medium was estimated. The determination was carried out by a disc method. After immersing a disc into coagulation water or into a standard solution of an antituberculosis drug, it was placed onto the surface of a modified Sauton agar medium to which was added the type strain (ATCC 23366) of *Mycobacterium aurum*. After incubation for 3 days, the diameter of a growth-inhibitory zone was measured and compared with standard curves. The modified Sauton agar medium contained sodium glutamate for asparagine. The indicator strain was cultivated in

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[1] From the National Chubu Hospital, Obu, Aichi, Japan.

[2] Requests for reprints should be addressed to Dr. Michio Tsukamura, The National Chubu Hospital, Obu, Aichi, Japan 474.

10 ml of Dubos liquid medium for 3 days, and this was added to 200 ml of the Sauton agar medium. The agar medium containing the indicator bacteria was dispensed in 10-ml aliquots into petri dishes.

Diagnosis of Lung Disease

The diagnosis was made using Tsukamura's criteria (16). In patients with new cavitary lesions: 2 or more isolations of the *M. avium* complex by monthly sputum examination in the first 3 months after admission, plus one or more isolations by daily sputum examination begun immediately after admission. Subsequently, 3 or more isolations of the *M. avium* complex demonstrated together with evidence of cavitary lesions and clinical symptoms (cough, sputa and/or fever). In patients with sclerotic cavitary lesions: 3 or more isolations of the *M. avium* complex by monthly sputum examination together with deterioration of clinical symptoms. Of the above isolates, at least one should have more than 100 colonies on the isolation medium.

Chemotherapy and Bacteriologic Response

Unless specially noted, the administration of antituberculosis drugs was carried out as follows: Rifampin, 0.45 g daily; isoniazid, 0.3 or 0.4 g daily; ethambutol, 0.75 or 1.0 g daily; ethionamide or prothioamide, 0.4 g daily; cycloserine, 0.5 g daily; pyrazinamide, 1 g daily; sulfadimethoxine, 1 g daily; kitasamycin, 1,200 mg (3 × 1) or 600 mg (1 × 1) daily; minocycline, 100 mg daily; streptomycin sulfate, 2 g weekly (1 g per day, intramuscularly); kanamycin sulfate, 3 g weekly (1 g per day, intramuscularly); enviomycin sulfate, 3 g weekly (1 g per day, intramuscularly).

Bacteriologic response was shown as follows: (*1*) negative conversion: a negative culture was continuously observed for a period of 6 months or more by monthly sputum examination; (*2*) intermittently positive: culture-positive in 2 to 4 specimens of the monthly sputum examinations made for 12 months; (*3*) frequently positive: culture-positive in 5 to 9 specimens of the monthly sputum examinations made for 12 months; (*4*) continuously positive: culture-positive in 10 to 12 specimens of the monthly sputum examinations made for 12 months.

Results

The Activities of Antituberculosis Drugs in Ogawa Egg Medium

The activities of many antituberculosis drugs in the coagulation water remaining in the bottom of tubes after sterilization of medium were lower than in those added to the medium before sterilization. These decreases are considered to be due to absorption to the coagulated protein and suggest that the activities are lower in the medium. Such decrease of the activity was most marked in rifampicin and then in kanamycin and enviomycin. No decrease occurred in ethambutol and isoniazid (table 1). The decreases should be considered in evaluating the data of the present study.

TABLE 1

ABSORPTION OF ANTITUBERCULOSIS DRUGS TO COAGULATED PROTEIN OF OGAWA EGG MEDIUM*

Agent	A. Concentration of Drug Added to Medium (μg/ml)	B. Concentration of Drug Found in Coagulation Water (μg/ml)	Ratio of A to B
Streptomycin sulfate	20	5	4:1
Kanamycin sulfate	100	15	6:1
	50	8	6:1
	25	4	6:1
Enviomycin sulfate	100	16	6:1
	50	7	6:1
	25	4	6:1
Ethambutol	5	5	1:1
	2.5	2.5	1:1
Rifampicin	20	1.3	15:1
	10	0.63	16:1
Isoniazid	40	40	1:1
	20	20	1:1
Minocycline	10	6.6	1.5:1
	5	3.2	1.5:1
Sulfadimethoxine	20	5	4:1
Kitasamycin	100	20	5:1
	20	5	4:1

* The activity of ethionamide could not be measured by the method used.

Estimation of Errors Involved in the Determination of Minimal Inhibitory Concentrations

As shown in table 2, except for ethionamide, experimental errors involved in the determination of MIC did not exceed beyond 3 steps (8-fold). It did not exceed 2 steps (4-fold) in the determination in kanamycin and enviomycin. The results show that an increase of the MIC values beyond 3 steps (8-fold) or in the case of kanamycin and enviomycin 2 steps (4-fold) is regarded as a real increase of the MIC caused by a factor other than the experimental errors.

Increase of Minimal Inhibitory Concentrations after Chemotherapy with Antituberculosis Drugs

Comparison of the MIC values estimated on different dates in the same patients is shown in table 3. Increase of the MIC values beyond the experimental errors was observed in Patients 1, 4, and 5 with

TABLE 2

ERRORS INVOLVED IN THE DETERMINATION OF MINIMAL INHIBITORY CONCENTRATION OF ANTITUBERCULOSIS DRUGS AGAINST TWENTY STRAINS OF *MYCOBACTERIUM AVIUM* COMPLEX*

Antituberculosis Drug	Errors Involved in Determining Minimal Inhibitory Concentration by Twofold Dilution Technique*	Upper Limit of Error with 95% Confidence†
Rifampicin	−0.11 ± 1.28	2.45
Streptomycin sulfate	−0.20 ± 1.05	1.90
Ethionamide	+0.35 ± 1.38	3.11
Isoniazid	+0.36 ± 1.01	2.38
Ethambutol	+0.45 ± 1.05	2.55
Kanamycin sulfate	−0.15 ± 0.74	1.33
Enviomycin sulfate	−0.20 ± 0.89	1.58
Sulfadimethoxine	+0.15 ± 1.18	2.51
Kitasamycin	−0.20 ± 1.19	2.18
Minocycline	−0.10 ± 1.41	2.72

* Minimal inhibitory concentrations of antituberculosis drugs were determined twice on each of 20 strains. Comparing the minimal inhibitory concentrations estimated twice on the same strain, differences between the first and the second determinations were scored as follows: The same levels in two determinations, score 0; the second was one step (twofold) higher than the first, score + 1; the second was two steps (fourfold) lower than the first, score − 2. The distribution of these scores could be regarded as distributed normally around the score 0. Values are mean ± SD.

† The data show that, except the error involved in the determination of the minimal inhibitory concentration of ethionamide, the estimation errors do not exceed 3 steps (8-fold).

TABLE 3

COMPARISON OF THE SUSCEPTIBILITY OF *MYCOBACTERIUM AVIUM* COMPLEX STRAINS BEFORE AND AFTER TREATMENTS WITH ANTITUBERCULOSIS DRUGS

Patient No.	Date	Minimal Inhibitory Concentration of Antituberculosis Drug (μg/ml)									
		RFP	SM	TH	INH	EB	KM	EVM	SX	KT	MC
1	April 1979	25	50	50	12.5	25	100	100	25	> 50	> 50
	February 1981	> 200	100	100	200	12.5	> 400	100	50	> 50	> 50
2	October 1979	> 200	100	> 200	100	25	100	100	50	> 50	25
	June 1980	> 200	100	> 200	> 200	50	200	400	50	> 50	50
	November 1980	> 200	100	100	200	50	200	200	50	> 50	50
3	March 1983	> 200	100	100	12.5	50	50	50	50	> 50	> 50
	July 1986	> 200	200	100	12.5	25	100	100	50	> 50	> 50
4	November 1979	> 200	> 200	200	12.5	50	> 400	100	> 50	> 50	50
	July 1986	> 200	> 200	> 200	50	50	> 400	> 400	50	50	> 50
5	September 1973	> 200	> 200	> 200	50	3.13	25	50	> 50	25	3.13
	June 1986	> 200	> 200	200	200	12.5	50	100	> 50	> 50	> 50
6	November 1980	> 200	> 200	> 200	> 200	12.5	100	100	> 50	> 50	50
	July 1986	> 200	> 200	> 200	> 200	50	100	50	> 50	> 50	25
7	November 1980	> 200	12.5	50	> 200	25	100	100	> 50	> 50	25
	June 1986	> 200	12.5	50	> 200	12.5	100	100	> 50	50	50

Definition of abbreviations: RFP = Rifampicin; SM = Streptomycin sulfate; TH = Ethionamide; INH = Isoniazid; EB = Ethambutol; KM = Kanamycin sulfate; EVM = Enviomycin sulfate; SX = Sulfadimethoxine; KT = Kitasamycin; MC = Minocycline.

respect to rifampin, isoniazid, kanamycin, enviomycin, and minocycline. In Patient 1, rifampin, isoniazid, and kanamycin were used for 24 months, and the MIC values for these drugs increased. In Patient 4, enviomycin was used for 10 months, and in Patient 5, minocycline was used for 24 months (table 4). The increase of the MIC values was regarded as the result of clinical use of these agents.

All 7 patients in table 3 were treatment-failure cases. Of these, except for Patient 1, the strains were originally highly resistant to many antituberculosis drugs.

Relationship between Negative Conversion and Susceptibility

The susceptibility of the *M. avium* complex strains to some antituberculosis drugs seemed to correlate with the bacteriologic response (tables 5 and 6). Nine patients, in whom the negative conversion of sputum culture was observed, showed frequently low MIC values to antituberculosis drugs, and they were treated by regimens including the drugs to which the *M. avium* complex strains were relatively susceptible.

Discussion

In the present study, the efficacy of antituberculosis drugs has been demonstrated in 2 ways. (*1*) Significant increase of the MIC values was observed after administration of the drugs (table 3). We believe the MIC shifts are due to suppression of relatively susceptible bacteria. Even if the blood or tissue concentration of a drug does not reach the MIC, it can select for resistant mutants by delaying the growth rate of susceptible bacteria and increasing the proportion of resistant bacteria (17). Previously, Ahn and associates (7) stated that the choice of initial chemotherapy is important in the treatment of the *M. avium* complex, because if it is inadequate to cause negative conversion, emergence of a more resistant population can occur. This sequence has been demonstrated in the present study (2). A correlation of the susceptibilities of the *M. avium* complex strains to the therapeutic effect has been shown in this study. Relative susceptibilities have often correlated to the negative conversion of sputum culture. Horsburgh and coworkers (11) reported that the response to antituberculosis drugs of patients with the *M. avium* complex correlated with

TABLE 4

TREATMENTS BY ANTITUBERCULOSIS AGENTS CARRIED OUT BETWEEN THE FIRST AND THE LAST EXAMINATIONS OF *MYCOBACTERIUM AVIUM* COMPLEX STRAINS*

Patient No.	Age (yr)	Sex	Treatment and Clinical Course
1	50	M	RFP + INH + KM, 24 months; intermittently culture-positive (8/24) by monthly sputum examination
2	65	F	RFP + INH + EB + EVM, 13 months; continuously culture-positive (10/13)
3	57	F	RFP + INH + KM, 2 months; RFP + MC + SX + KT (600 mg daily), 26 months; RFP + KM + MC + SX + KT (1200 mg daily), 12 months; continuously culture-positive (39/40)
4	62	M	EB + TH + INH, 14 months; RFP + EVM + MC, 10 months; RFP + MC + SX + KT (600 mg daily), 10 months; RFP + EB + MC + SX + KT (1,200 mg daily), 18 months; ofloxacin (0.3 g daily) + MC + SX, 9 months; RFP + MC + SX + KT (1,200 mg daily), 12 months; INH + PZA + TH + MC + KT (1,200 mg daily), 7 months; continuously culture-positive (76/80)
5	41	M	SM + INH, 12 months; EB + INH + SX, 12 months; RFP + INH + SX, 15 months; INH + SX, 18 months; INH, 24 months; EB + SX + KT (400 mg daily), 6 months; MC + SX + KT (400 mg daily), 24 months; (between these treatments, there were periods of no treatment for 42 months); intermittently culture-positive (26/153)
6	73	M	RFP + INH + SM, 11 months; EB + TH, 12 months; RFP + INH + MC + SX + KT (1,200 mg daily), 16 months (between these treatments, there were periods of no treatment for 29 months; treatments during hospitalization were frequently culture-positive (16/39)
7	58	M	INH, 24 months; RFP + MC + SX + KT (600 mg daily), 24 months; MC + SX, 19 months; intermittently culture-positive (15/67)

For definition of abbreviations, *see* table 3.

* Bacteriologic response is shown by monthly sputum examination. The age is that in the first examination.

TABLE 5

THE SUSCEPTIBILITY OF *MYCOBACTERIUM AVIUM* COMPLEX STRAINS FROM PATIENTS AT THE BEGINNING OF TREATMENT

Patient No.	Minimal Inhibitory Concentration of Antituberculosis Agent (μg/ml)										Bacteriologic Response (Culture Positivity)*
	RFP	SM	TH	INH	EB	KM	EVM	SX	KT	MC	
8	1.6	50	25	6.25	3.13	100	50	25	25	12.5	(−)
9	12.5	100	50	6.25	25	50	50	> 50	> 50	6.25	(−)
10	12.5	12.5	12.5	6.25	6.25	50	25	50	> 50	50	(−)
11	6.25	12.5	50	1.6	12.5	25	12.5	12.5	3.13	0.8	(−)
12	6.25	12.5	> 200	50	12.5	25	50	50	25	3.13	(+)
13	12.5	50	12.5	3.13	12.5	50	50	25	12.5	3.13	(+)
14	3.13	12.5	50	12.5	12.5	50	25	> 50	50	6.25	(+)
15	6.25	50	100	12.5	12.5	200	50	50	25	6.25	(+ + +)
16	> 200	25	25	1.6	6.25	50	50	12.5	50	50	(−)
17	1.6	12.5	25	6.25	12.5	12.5	25	12.5	25	12.5	(−)
18	3.13	> 200	25	100	6.25	> 400	25	12.5	> 50	25	(−)
19	25	100	200	200	50	100	200	> 50	> 50	> 50	(−)
20	> 200	12.5	> 200	> 200	25	25	25	> 50	> 50	25	(+ +)
21	100	50	25	3.13	12.5	25	50	12.5	> 50	50	(−)
22	> 200	50	> 200	> 200	50	25	100	> 50	> 50	> 50	(+ + +)
23	> 200	100	200	200	> 50	100	100	25	> 50	> 50	(+ + +)
24	> 200	100	100	50	50	100	100	50	> 50	50	(+ + +)
25	> 200	12.5	100	50	12.5	25	25	25	> 50	50	(+ + +)
26	200	25	50	100	12.5	50	25	12.5	12.5	6.25	(+ + +)

For definition of abbreviations, *see* table 3.
* See text for bacteriologic responses.

TABLE 6

TREATMENTS AND CLINICAL COURSE OF PATIENTS SHOWN IN TABLE 5

Patient No.	Age* (*yr*)	Sex	Treatments and Clinical Course
8	61	F	RFP + EB + INH, 2 months; RFP + INH, 4 months; RFP + KM, 11 months; negative conversion was achieved by the first regimen and continued for the observation period
9	62	M	RFP + EB + INH, 5 months; negative conversion
10	65	M	RFP + EB + TH + INH, 6 months; negative conversion
11	57	M	RFP + EB + INH + PZA, 6 months; negative conversion
12	55	M	RFP + INH + KM + TH + CS, 2 months; RFP + KM + INH, 10 months; intermittently culture-positive (4/12)
13	45	F	RFP + INH + SM, 4 months; MC + SX + KT (600 mg daily), 24 months; intermittently culture-positive: first 4 months (3/4) and the latter 24 months (4/24)
14	49	F	RFP + INH, 18 months; intermittently culture-positive (5/18)
15	62	F	INH, 10 months; continuously culture-positive (10/10) (an obvious error in treatment)
16	64	M	RFP + INH + SM, 6 months; negative conversion was achieved after 2 months
17	36	F	RFP + EVM + INH, 3 months; RFP + INH + KM + MC, 10 months; negative conversion was achieved after 4 months and continued for 9 months
18	20	M	RFP + INH + SM, 6 months; negative conversion was achieved after 3 months and cavity disappeared
19	42	M	RFP + INH + SM, 11 months; negative conversion occurred after 4 months and cavity disappeared
20	72	M	RFP + INH + EB, 24 months; EB + INH, 18 months; frequently culture-positive (21/42)
21	62	M	RFP + INH + EB + MC, 14 months (Before chemotherapy, 5 daily sputum examinations showed 5 positive cultures with more than 100 colonies on the isolation medium. After chemotherapy, however, the number of colonies decreased to less than 10, and after 8 months, negative conversion occurred; the negativity of cultures was confirmed for 7 months)
22	63	M	RFP + INH, 6 months; continuously culture-positive (5/6)
23	63	M	RFP + EB + MC, 4 months; continuously culture-positive (4/4) and died because of respiratory failure
24	67	M	EVM + INH + RFP + MC + SX + KT (1,200 mg daily), 24 months; continuously culture-positive (19/24)
25	32	M	RFP + INH + SM, 5 months; continuously culture-positive (5/5)
26	62	M	RFP + EB + INH, 6 months; continuously culture-positive (6/6)

For definition of abbreviations, *see* table 3.
* Ages are those at the beginning of treatments.

the *in vitro* susceptibility testing. They stated that the *M. avium* complex strains often were susceptible to rifampin and ethambutol. In our study, too, relative susceptibilities to these drugs seemed to correlate with the negative conversion of patients. Furthermore, our findings suggest that the outcome of patients is greatly influenced by the susceptibility of the strain that has caused infection, even though it is possible that the outcome is also influenced by the host condition (18, 19).

In addition, we would like to draw attention to the possibility that the susceptibility of the *M. avium* complex strains may be different according to the area where the strains are isolated. We observed previously, testing the same method, that the strains isolated in Japan were often more resistant to rifampin than the strains isolated in the United States (20).

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