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Resistance to rifampicin and isoniazid in strains of *Mycobacterium tuberculosis*

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SUMMARY Drug susceptibility studies on strains of *Mycobacterium tuberculosis* isolated from widely different populations of patients and tested by two different techniques indicated that all 55 strains resistant to rifampicin were also resistant to isoniazid, while many strains resistant to isoniazid were found to be susceptible to rifampicin. This observation, which has as yet unknown laboratory and clinical significance, may be particularly useful in management of patients. Further studies are called for to establish this relation.

Rifampicin is a recent addition among antituberculous drugs. It is considered to be the most valuable and effective chemotherapeutic agent after isoniazid and is now widely used in the first line of antituberculous drugs.

Since the discovery and characterisation of this drug much work has been done on its bactericidal activity, mechanism of action, and development of resistance.[1-4] It was noted that rifampicin, having a different chemical structure from isoniazid, has independent bactericidal action, and in wild strains of *M tuberculosis*, the incidence of rifampicin-resistant mutants was appreciably lower, while the serum level attained on standard oral dosage was much higher than isoniazid.[5] Moreover, the pattern of resistance development to rifampicin was reported to be a single step type without having any intermediate level of resistance.[3 5] Hobby (1968) compared the two drugs, isoniazid and rifampicin in vivo and in vitro and reported that on a weight basis rifampicin was approximately half as active as isoniazid against isoniazid-susceptible strains of *M tuberculosis*.[1] It has been reported that there is no antagonism between rifampicin and isoniazid, streptomycin, or ethambutol; and an absence of synergism between rifampicin and isoniazid has also been reported.[2 6]

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There is little information concerning the properties of rifampicin-resistant strains. Hobby *et al.*[2] working with the H37Rv strain mentioned that "cells resistant to rifampin in vitro appeared to be fully susceptible to other antimicrobial agents." Published reports, in general, have made casual mention of the absence of cross resistance between rifampicin and isoniazid without actually presenting supporting data.[1 7]

The purpose of this report is to present our observations on rifampicin resistance among strains of *M tuberculosis* from two different studies in which all rifampicin-resistant strains were found to be isoniazid-resistant as well.

Material and methods

The subjects of the first study were tuberculous patients in Pakistan. The study included hospitalised patients as well as cases from outpatient clinics. The majority of these cases were chronic, while some were newly diagnosed patients with no history of previous treatment. The patients were tested at the Pakistan Medical Research Council's Tuberculosis Research Center, Mayo Hospital, Lahore.

A total of 1063 strains from the same number of patients, after identification as niacin-positive *M tuberculosis*, were subjected to drug susceptibility testing by the standard proportion method using Löwenstein-Jensen (LJ) medium.[8] The strains were tested against drugs listed in Table 1.

The critical proportion for resistance—that is, the percentage of resistant bacteria among a test

Table 1 *Selection of drugs and their concentrations for susceptibility testing*

Drug	*Concentration (μg/ml LJ medium)*
Isoniazid (INH)	0·2
Streptomycin (SM)	4·0
p-aminosalicylic acid (PAS)	0·5
Ethionamide (ETA)	20·0
Ethambutol (EMB)	2·0
Thiacetazone (THA)	2·0
Rifampicin (RMP)	40·0

population beyond which the strain is considered resistant, was 1% for INH, RMP, PAS and EMB while for SM, ETA, and THA it was 10%.

In another study, drug susceptibility was tested by an entirely different radiometric method in which $^{14}CO_2$ was measured as an index of growth in 7H12 medium containing 1 μCi/ml 1-^{14}C-palmitic acid.[9] A total of 300 isolates of *M tuberculosis* were provided by the Center for Disease Control (CDC), Atlanta. Drug susceptibility tests were done by the conventional plate method at CDC while the rapid radiometric method was employed at the University of Maryland. These cultures were tested against isoniazid, rifampicin, streptomycin, PAS and ethambutol.

Results

Among 1063 patients who were studied in Pakistan, 200 cases gave no history of previous treatment and all the isolated strains from these patients were found to be susceptible to rifampicin (40 μg/ml in LJ medium), although isoniazid resistance was not infrequent among these cases. Among 863 chronic cases (mostly long treated), 21 were found to be excreting populations resistant to rifampicin. It was noted that all 21 of these strains were also resistant to isoniazid (0·2 μg/ml LJ medium), although not uniformly resistant to any other drug tested (see Table 2). Only six of 13 whose previous drug treatment was known had histories of treatment with rifampicin.

In the CDC study, 34 strains were found to be resistant to rifampicin (1 μg/ml of 7H12 medium). All these were found to be resistant to isoniazid as well (0·2 μg/ml of 7H12 medium). There was 95% agreement of these results with drug testing done independently on 7H10 medium on the same cultures at the Center for Disease Control.

Discussion

Rifampicin has been introduced only recently in Pakistan, and, due to its high cost, is not widely used, least of all among poor patients. Out of 21 rifampicin-resistant cases, only six had a history of treatment

Table 2 *Analysis of cases with rifampicin resistance*

No	*History of previous treatment*	*Resistance to other drugs*
1	INH SM PAS EMB PZA	INH SM
2	INH SM PAS EMB PZA	INH SM EMB
3	INH SM PAS EMB ETA RMP	INH SM ETA EMB
4	INH SM PAS EMB THA PZA RMP	INH SM EMB
5	INH SM PAS THA PZA	INH SM PAS
6	Chronic treated case	INH SM PAS ETA EMB
7	Chronic treated case	INH THA
8	INH SM PAS	INH
9	Chronic treated case	INH SM PAS
10	Treatment status not known	INH SM
11	INH SM PAS ETA EMB THA PZA PMP	INH SM ETA
12	Chronic treated case	INH SM
13	INH SM PAS ETA EMB THA CYCL PYZ RMP	INH SM PAS EMB
14	Chronic treated case	INH SM EMB
15	Chronic treated case	INH SM
16	INH SM THA	INH
17	Chronic treated case	INH
18	INH SM PAS ETA EMB RMP	INH SM EMB
19	INH SM	INH
20	INH EMB PZA RMP	INH SM
21	INH SM PAS THA	INH SM PAS THA

INH = isoniazid, SM = streptomycin, ETA = ethionamide, EMB = ethambutol, THA = thiacetazone, RMP = rifampicin, PZA = pyrazinamide, CYCL = cycloserine, PAS = *p*-aminosalicylic acid.

with rifampicin. It was interesting to note that all these rifampicin-resistant cases had had various combinations of other antituberculous drugs and had developed resistance against other drugs as well, but apart from isoniazid, there was no consistent pattern of resistance against any other drug.

The second observation was drawn out of an altogether independent study concerned with development of a radiometric method for drug susceptibility determination. The cultures were from patients' sputa which were submitted from all over the United States. Since this was a comparative study of two different methods of drug susceptibility testing, no information is available concerning the past history of treatment of these patients. However, it is interesting to note that all the 34 rifampicin strains were consistently resistant to isoniazid while this consistency was not observed in the case of any other drug.

In any event, with two different methods of testing for two quite different patient populations, it was interesting to find all rifampicin-resistant cases also to be yielding strains resistant to 0·2 μg isoniazid/ml medium. This observation has some clinical significance. It would be interesting to investigate whether simple prolonged antituberculous treatment and emergence of resistance against other drugs are some of the predisposing factors in the emergence of spontaneous rifampicin resistance. Selection of

rifampicin-resistant mutant strains of *M tuberculosis* in vitro does not result in simultaneous mutation to isoniazid resistance: the rifampicin (250 μg/ml) resistant mutant of H37Rv from the Trudeau Mycobacterial Collection (No 331) is susceptible to 0·2 μg isoniazid/ml of 7H12 medium. Nevertheless, whatever the mechanism or mechanisms involved, it would appear that resistance to rifampicin in vivo usually emerges along with resistance to isoniazid and that the rifampicin resistance seems to arise after multiple drug treatment in which rifampicin itself was not necessarily included; the association of resistance against both of the most effective drugs used in tuberculosis treatment has very important implications for the management of patients. It should be pointed out that strains in these two studies were not tested in a medium containing both drugs together (to find out what proportion of the bacterial population was resistant to the drug combination).

Finally, it has been reported that a rifampicin-resistant mutant strain, selected in vitro, was markedly less virulent for guinea pigs than the parent rifampicin-susceptible strain.[10] No note was made of the isoniazid susceptibility or resistance or the catalase activity of this strain. Since rifampicin is such a valuable new drug, it would be worthwhile to investigate further the associative features of rifampicin and other antimycobacterial drugs.

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