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Effect of Rifampin on Cutaneous Hypersensitivity to Purified Protein Derivative in Humans

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The effect of rifampin on cutaneous hypersensitivity was studied in 11 tuberculous patients. A suppressive effect was noted in eight patients, five of whom developed a negative response to the strength of purified protein derivative to which they had been shown to be previously sensitive. In one instance complete anergy to purified protein derivative was noted. It appears that rifampin has immunosuppressive effects in some patients when used in conventional doses.

Rifampin has been widely used for the treatment of tuberculosis. Side effects which have been reported with rifampin include gastrointestinal disturbances, such as nausea, vomiting, and diarrhea; liver damage, primarily reflected as an elevation of hepatic enzymes; and hematopoetic disturbances, usually occurring as reversible leukopenia and thrombocytopenia (17, 20). All of these adverse reactions have been uncommon, and the drug has been an important addition to mycobacterial chemotherapy.

Another side effect of rifampin of considerable interest has been noted. Daddi (Abstr. 11th Congr. Intern. Med., p. 162, 1970), Nilsson (18), Paunescu (19), Dajani et al. (4), and Grassi and Pozzi (10) have all reported that rifampin has immunosuppressive properties in various animal test systems. Of particular interest has been the observation that suppression of skin reactivity to purified protein derivative (PPD) in immunized guinea pigs occurred when rifampin was administered to these animals (10), and this suppressive effect disappeared after cessation of the drug (Abstr. 11th Congr. Intern. Med., p. 162, 1970).

Recently Graber and co-workers (9) have reported that rifampin, administered in usual clinical doses to tuberculous patients, was associated with light-chain proteinuria. The drug was also noted to interfere with antibody response to keyhole limpet hemocyanin and suppressed the anamnestic antibody response to *Salmonella typhi* vaccine.

Taken together, these reports indicate that rifampin can have significant immunosuppressive reactivity in animals and, in some instances, in humans. The study reported here was designed to see if this drug also had a similar immunosuppressive effect on delayed-type hypersensitivity in humans by using as an assay the cutaneous reaction to PPD in individuals infected with mycobacteria.

MATERIALS AND METHODS

Patients. The subjects of the study were patients of both sexes referred to the tuberculosis unit of the University of Iowa Hospitals. All of the patients who received rifampin as part of their anti-tuberculosis therapy, but who were not on steroids and who had been observed for 2 months or longer, were included in the study. Eleven patients were included in the study, 8 males and 3 females ranging in age from 15 to 77 years. Nine were infected with drug-resistant *Mycobacterium tuberculosis* and two with *Mycobacterium kansasii*. All had far advanced disease and their sputa contained mycobacteria which were resistant to isoniazid when tested in vitro. Base line skin tests were obtained on admission. All had initial skin test reactions to PPD-S equal to or greater than 10 mm in diameter to either 1 or 5 USP U. Other routine studies such as hemoglobin, white blood cell count, differential white blood cell count, hematocrit, urinalysis, and liver function tests were also obtained initially and 2 weeks after beginning rifampin. Subsequent determinations were obtained monthly while the patient was hospitalized. All the patients demonstrated clinical improvement shortly after therapy was initiated with the drug, as evidenced by weight gain, improved well being, diminished cough, improvement in their chest X ray, and gradual disappearance of the infecting

organisms from the sputum. All of the patients did well during the course of study, as based on the usual clinical criteria; none died, and none were severely ill from any cause during the study. Toxicity to rifampin, primarily nausea and vomiting requiring temporary cessation of therapy and usually for 1 or 2 days, was encountered in occasional patients. Liver toxicity necessitated a 4-week interruption of therapy in one case, but all others continued to receive the drug during the course of this study. None were leukopenic prior to or during the study.

The purified protein derivative of tuberculin obtained from *M. tuberculosis* (PPD-S) stabilized with 0.005% sorbitan monoleate (Tween 80) produced by Connought Medical Research Laboratories (Willowdale, Ont., Canada) was used for these studies. The skin test was applied in the usual manner on the flexor surface of the forearm and read 48 h after application, the induration was recorded in millimeters.

All of the patients were tested prior to therapy, and the skin test was repeated 7, 14, and 30 days after rifampin therapy was instituted. Subsequent skin tests were obtained, at an appropriate dilution, monthly thereafter.

Drugs. All of the study patients received 600 mg of rifampin (Rifadin, Dow Chemical Corp., Indianapolis, Ind.) administered once daily throughout the period of observation, except where noted above.

All of the patients were also treated with other antituberculous drugs during the course of the study. In all instances isoniazid and ethambutol were used. Kanamycin, streptomycin, and capreomycin were also used on some occasions. Other drugs were given during the course of this study to some of the patients. Included among these drugs were such substances as digoxin, multivitamins, barbiturates, and various tranquilizers.

To see what effect antituberculous drugs, other than rifampin, had on cutaneous tuberculin sensitivity, 10 patients, all with far advanced or moderately far advanced pulmonary disease who had sputa that was positive on culture for isoniazid sensitive strains of *M. tuberculosis*, were selected at random and tuberculin tested prior to therapy with isoniazid, ethambutol, and streptomycin in conventional doses. These patients were retested on a single occasion after 2 to 6 months of treatment.

RESULTS

The effect of rifampin administration on skin test reactivity is shown in Table 1. Skin reactivity to PPD declined in most instances. Of the 11 patients included in the study, eight had reduction of the induration produced by this antigen. In five instances the subjects developed a negative response to the strength of tuberculin to which they had been shown to be sensitive on several occasions in the past. Three of these patients were retested with a higher concentration of PPD (5 USP U) and were found to be positive to this concentration, but subsequently demonstrated a progressive decline in reactivity (patients 6, 7, and 8). Patient 8 eventually became anergic to 250 USP U after 15 months of therapy.

It was also noted that three of the patients

TABLE 1. *Skin test reactivity to PPD after rifampin therapy*[a]

Group	Patient no.	Intradermal reaction in mm of induration according to treatment time								
		Prior to treatment	7 days	14 days	1 mo	2 mo	3 mo	6 mo	10–12 mo	Remarks
I. 1 USP U	1	22	16	14	14	13		13	11	
	2	35	43	27	34	37	25	10		
	3	10	9	12	14	20	20	23		
	4	17	20	10	10	10	20	13	5	Rifampin discontinued for 4 weeks at 2nd mo
	5	10	10	5	5	0				
	6	16	5	7	0		0 (15)	0 (6)		
	7	22	8	12	17	0	0 (23)	0 (19)	0 (0)	*M. kansasii* infection
	8	10	12	12	0	0	0 (11)	0 (0)	0 (0)	Negative to 250 USP U at 15 mo
Mean ± SD		17.7 ± 8.1	15.4 ± 11.2	12.4 ± 5.6	11.7 ± 10.1	11.5 ± 12.6		9.3 ± 7.4		
II. 5 USP U	9	23	23	35	23	22		—	—	
	10	35	35	30	15	16		—	—	
	11	21	15	8	5	0		—	—	*M. kansasii* infection
Mean ± SD		24.2 ± 7.1	24.5 ± 8.2	24.3 ± 18.8	14.3 ± 7.7	12.7 ± 9.2				

[a] —, Not done; SD, standard deviation. Numbers in () indicate results when tested with 5 USP U.

who had received long-term rifampin therapy had no significant reduction in their reactivity (patients 2, 3, and 9) at 2 months, and only one of these patients (patient 2) had a modest suppression of his reactivity when tested on one occasion after 6 months of therapy. Indeed, patient 3 had an increase in PPD sensitivity during the course of the study, as was indicated by a larger area of induration. Patient 9 was remarkably consistent with no significant change in his reactivity during 2 months of rifampin therapy.

Statistical analysis of this data, by using simple regression analysis of each patient, indicated that the decline in skin reactivity was significant at the 0.05 level (11).

One other point should be noted. While on therapy patient 4 demonstrated a mild, consistent suppression of her reaction to PPD which persisted until the 2nd month. At that time rifampin was discontinued for 4 weeks when she developed mild liver toxicity. After the rifampin had been discontinued for 4 weeks, the induration produced by 1 USP U of PPD-S had returned to its pretreatment diameter. When rifampin was restarted, a decline in reactivity ensued and persisted over the next 9 months.

Patients not receiving rifampin, but treated with other antituberculous drugs, were retested after 2 to 6 months of therapy and were found to have no significant change in mean skin reactivity (Table 2). Although this group was not tested as frequently as the experimental group, it indicated that under the conditions used, therapy with antituberculous drugs, including ethambutol but not rifampin, was not accompanied by a decline in delayed-type hypersensitivity as measured by cutaneous reactivity to PPD.

TABLE 2. *Skin test reactivity to PPD in patients not receiving rifampin*

Patient no.	Reaction in mm of induration	
	Prior to treatment	After 2 mo to 6 mo of therapy
1	9	23
2	15	18
3	25	18
4	10	58
5	23	20
6	22	22
7	18	15
8	25	25
9	14	8
10	9	23
Mean ± SD[a]	17.0 ± 6.2	23.9 ± 12.6

[a] SD, Standard deviation.

DISCUSSION

The reaction to intradermal tuberculin has been considered to be a model example of the delayed-type, cell mediated immunological response. Because of the frequent clinical use of this skin test, it has been the most widely studied example of delayed-type of hypersensitivity.

It has been shown that this reaction can be affected by a number of factors. Age (12), a serious illness (2), hypothyroidism (21), severe or disseminated tuberculosis or tuberculous pleural effusions (8, 13), Hodgkin's disease (23) and sarcoidosis (1, 16) all have been shown to be associated with a diminished response to tuberculin.

Drugs that suppress immunity, such as steroids, can also suppress skin test reactivity in humans (15, 21) and there has been an increasingly long list of materials that can interfere with the sequence of events involved in cell-mediated immunity and that can interfere with the tuberculin reaction, both in animals and humans.

It appears from the data presented here that rifampin may be added to the list of drugs that can suppress skin test reactivity to PPD in man in some instances. A consistent, progressive decline in the induration produced by PPD-S was noted in many of the individuals who received the drug in the usual clinical dose. Only a minority of the patients who received rifampin failed to respond. Although almost all of the patients did not become anergic to tuberculin, in one instance a patient became nonreactive to 250 USP U after receiving the drug for several months. In another instance cessation of rifampin in an individual resulted in a return of the skin test to its pretreatment level.

These results do not agree with the results reported by Graber et al. (9), who reported that skin test reactivity to PPD-S had not been suppressed in patients on rifampin. The details of this negative study were not described, and speculation about the causes of this discrepancy between this report and the results noted here should be withheld. It might be mentioned, however, that a short-term study might fail to note a rifampin effect, for it appears that in many instances several weeks had ensued before a suppressive effect could be demonstrated and that the effect is only partial in almost all instances where it was encountered.

Because the patients used in this study also

received other antituberculous drugs, their effect on PPD reactivity should also be considered. There have been several studies of the effects of chemotherapy on the tuberculin reaction. Human studies noting the effects of isoniazid on skin test reactivity in individuals with chronic pulmonary tuberculosis have not demonstrated a clear suppressive effect. Saloman and Angel (22), in reporting a study of the effect of adrenocorticotropin on skin test reactivity, used as controls 30 patients on isoniazid, para-aminosalacylic acid, and streptomycin, and noted no change in skin reactivity in this control group during a 6-week period. Other investigators, in similar studies of the effect of chemotherapy on skin test reactivity in tuberculous patients, found little (14) or no effect (7).

Because the effect of ethambutol, administered in combination with isoniazid and streptomycin, on skin test reactivity could not be found in the literature, a small group of patients on such therapy was also tested. This group of patients on isoniazid, streptomycin, and ethambutol in conventional doses, but not on rifampin, did not have a significant decline in skin test reactivity during a 2- to 4-month period. Although this group was not skin tested as frequently as the study population, conditions of the two groups were comparable, except that the patients receiving isoniazid, ethambutol, and streptomycin were infected with drug-sensitive strains of mycobacteria and received a lower dose of INH and pyridoxine.

Another difference between the two groups and another explanation of the results noted in patients receiving rifampin presented here should also be considered. Duboczy (6) reported that repeated skin testing in the same area of skin accelerated the tuberculin reaction. As a result, repeated skin testing in the same area of skin could give a false impression of a diminished reaction if always measured at 48 h. Because this problem might be a concern in the rifampin-treated patients reported here, it should be indicated that although skin testing was repeated, it was in random locations. In addition, after this report came to our attention, two patients, who were part of the study population, were retested, simultaneously in both arms. The reactions in each arm were within 3 mm in diameter of each other at 48 h, in both cases.

The effect of rifampin on the skin test reaction to PPD in humans reported here was compatible with the results obtained with this antibiotic in animals, both specifically with reference to the suppression of the intradermal reaction to PPD in guinea pigs (5, 10) and generally in that rifampin can suppress immunological responses to a variety of antigenic stimuli (18, 19). Although the patients reported here received a lower dose of rifampin (approximately 10 mg per kg per day) than the dose required to suppress PPD reactivity in guinea pig studies reported by one group of investigators (20 mg per kg per day) (5), it was nearly the same as that found to be effective by others (10).

One last point should be considered. The patients used in this study were a selected group, for they all had a history of poor response to prior antituberculous chemotherapy or were infected with *M. kansasii*. It could be suggested that the poor response to their respective infections was the result of some sort of deficient immunological response and as a result they, as a group, were more susceptible to rifampin suppression than might be found in other patients who respond to infection more favorably. This question can be answered only by appropriate studies.

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ADDENDUM IN PROOF

Since this article was submitted for publication, Rubin et al. have reported (Abstr. Intersci. Conf. Antimicrob. Ag. Chemother., 13th, Washington, D.C., abstr. no. 6, 1973) impairment of skin hypersensitivity to PPD in tuberculous patients receiving 600 mg of rifampin per day after 12 weeks of therapy.

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