CLINICAL TRIAL OF P.A.S. SALT OF ISONIAZID IN TREATMENT OF PULMONARY TUBERCULOSIS

BY

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In 1953 Smith and Wiederkehr reported on the bacteriostatic properties against *M. tuberculosis* of a new drug then called GEWO 339. This is an additive compound between P.A.S. and isoniazid to which the following formula is attributed:

This substance was said to inhibit the growth of ordinary strains of tubercle bacilli at a concentration of 0.1 $\mu\text{g.}$ per ml. of medium. It was also claimed that strains which were resistant to isoniazid and P.A.S., either alone or in combination, though not as sensitive as normal strains to GEWO 339, were nevertheless still sensitive at therapeutic levels. Strains which grew normally on media containing 100 $\mu\text{g.}$ of isoniazid and also media containing 100 $\mu\text{g.}$ of P.A.S. were inhibited by 5 $\mu\text{g.}$ per ml. of GEWO 339. Brodhage, of the Institute of Bacteriology, Lucerne, confirmed these results in a personal communication.

The Investigation

In 1954 GEWO 339 became available in this country, and an opportunity presented itself to confirm the results of Smith and Wiederkehr and of Brodhage. For the sake of brevity the P.A.S. salt of isoniazid is referred to as the salt (Sa).

The technique of measuring the resistance of a number of strains of *M. tuberculosis* to Sa, P.A.S., isoniazid, and streptomycin used in this experiment is the standard technique described by the Medical Research Council (1953).

Fourteen strains were compared with the standard strain H37Rv. Of these strains, 10 were freshly isolated, but the remaining four were known to be resistant to either P.A.S. or isoniazid, or both. Of the remaining four strains, Nos. 8687 and 5300 were resistant to P.A.S. but sensitive to streptomycin, isoniazid, and Sa. Strain 9488 was fully sensitive to streptomycin, resistant to isoniazid and P.A.S., and, though still sensitive to Sa, was less sensitive than a normal strain, as slight growth occurred in a concentration of 1 $\mu\text{g.}$ per ml. of Sa. Strain 9371 was fully resistant to isoniazid and P.A.S., showed a slight loss of sensitivity to streptomycin, and a similar loss of sensitivity to Sa, as once again slight growth occurred in a concentration of 1 $\mu\text{g.}$ but not in 4 $\mu\text{g.}$ The results are set out in Table I.

These results were thought to be sufficiently encouraging to justify a clinical trial of Sa. On ethical grounds it was considered undesirable to treat new patients with Sa. As it seemed unlikely that the drug would prove of value in

patients infected with strains which were already resistant to streptomycin, isoniazid, and P.A.S., the following criteria for selection of patients for the trial were laid down. The patients should (1) have chronic pulmonary tuberculosis with one or more cavities and sputum strongly positive on two direct examinations within the last month; (2) be unsuitable for any treatment other than antibacterial drugs; (3) have had at least three months' treatment with isoniazid or P.A.S., either alone or together at some time in the past; (4) have had no change in treatment during the preceding two months—that is, drugs not started, changed or stopped, not newly confined to bed or allowed to get up at home, not admitted to or discharged from hospital; and (5) be likely to remain under the present regime for at least three months. These criteria were designed to reduce the chances of changes in sputum being due to other factors than the Sa. Patients with plentiful sputum were regarded as more suitable than those with only a trace. Out-patients were included.

In laying down these criteria it was appreciated that only very chronic and extensively diseased patients would be available. Therefore, judgment on clinical or radiological grounds would be impossible. It was hoped, however, that comparison between the pre-treatment and post-treatment strains in their sensitivity to P.A.S., isoniazid, and Sa would yield valuable information concerning the efficacy of Sa in the treatment of pulmonary tuberculosis.

Five chest centres co-operated in providing patients, and altogether a total of 25 patients were offered. Of these six were rejected on the ground that the amount of sputum was insufficient and could not always be obtained.

For the first three weeks of the trial the patients received no drug treatment. During this period two 24-hour specimens of sputum were submitted to the laboratory each week. At the end of the control period treatment was begun. Sa was given as tablets, each of which contained 100 mg. of active substance, and each patient received a daily dose of 600 mg., in three doses of 200 mg. This treatment continued for 12 weeks, and during the whole period two 24-hour specimens of sputum were submitted to the laboratory each week. All the patients included in the trial were suffering from bilateral chronic pulmonary tuberculosis. All showed cavitation, which was bilateral in the majority of cases. Unfortunately, one patient, a woman aged 60 with severe bilateral tuberculosis and a sedimentation rate of 80 mm. in one hour, died shortly after treatment began and has had to be excluded. In a second case, that of a woman aged 43 with a long history of gastric ulceration, the ulcer perforated and she was withdrawn from the trial shortly after treatment had begun. The remaining 17 cases completed the trial without any untoward incident.

Results

Each 24-hour specimen was concentrated and cultured on Löwenstein-Jensen medium for four weeks, after which the cultures were read. The growth in all cases was very heavy, and any patient whose sputum did not yield a confluent growth in the majority of pre-treatment specimens was excluded from the trial. Subsequently, when under treatment the positivity of the sputum diminished, the cultures were divided into the following groups: (1) confluent growth, where no individual colonies could be detected; (2) scanty growth, where there was less than a total of 10 colonies on a pair of slopes; (3) moderate growth, for all degrees of growth between scanty and confluent; (4) no growth.

Sensitivity tests were performed on two of the cultures from the control period, and at weekly intervals after treatment had begun. Each strain was inoculated on to slopes of Löwenstein-Jensen medium containing the following concentration of drugs: streptomycin, 3 $\mu\text{g.}$ and 15 $\mu\text{g.}$; isoniazid, 0.2, 1, and 5 $\mu\text{g.}$; P.A.S., 2, 10, and 100 $\mu\text{g.}$; and Sa, 0.2, 1, 5, and 50 $\mu\text{g.}$ Partial resistance was reported when growth occurred on 3 $\mu\text{g.}$ of streptomycin, 1 $\mu\text{g.}$ of isoniazid, 2 $\mu\text{g.}$ of P.A.S., and 1 $\mu\text{g.}$ of Sa. Strains growing on higher concentrations were recorded as resistant.

TABLE I.—Minimum Concentration Which Inhibited Growth

	Streptomycin	Isoniazid	P.A.S.	Sa
H37	0.06	0.06	0.06	0.06
8687	0.06	0.5	64	0.06
5300	0.25	0.25	64	0.25
9488	0.06	64	64	4
9371	4	64	64	4

TABLE II.—Results of Sensitivity Tests

Case No.	Sex and Age	Previous Treatment (Total Dosage in G.)				Pre-treatment					Post-treatment				
		St.	P.A.S.	Isoniazid	Duration Months	First 3 Cult's.	Sensitivities			Last 3 Cults.	Sensitivities				
							St.	P.A.S.	Isoniazid		Sa	St.	P.A.S.	Isoniazid	Sa
1	M 64	104	840	64	64	CCM	S	S	R	S	CMM	S	S	R	Pr
2	M 38	194	4,740	69	39	CCC	Pr	Pr	R	Pr	ScMM	R	Pr	R	Pr
3	M 42	+++	+++	+++	48	CCC	R	S	Pr	Pr	MMM	R	S	Pr	Pr
4	F 34	113	4,060	30	46	MCC	R	S	S	S	OScSc	R	S	Pr	S
5	F 20	40	3,200	54	72	MCC	R	S	R	S	MMM	Pr	S	R	S
6	M 49	136	10,020	42.6	48	MCM	S	S	R	S	MScM	R	Pr	Pr	S
7	M 51	224	2,800	28	21	CCC	R	S	S	S	ScMM	R	S	R	S
8	F 34	198	3,700	79	27	CCC	R	S	R	S	ScOSc	Pr	S	R	S
9	M 46	308	3,436	34.6	53	CCC	R	S	S	S	MMM	R	S	S	S
10	F 34	130	11,540	30.6	50	MCC	R	S	Pr	S	MMC	R	S	S	S
11	F 38	65	1,300	0	38	CCC	S	S	S	S	MMM	S	S	S	S
12	M 62	84	9,400	38	51	CCC	S	S	S	S	OOO	S	S	S	S
13	M 35	200+	3,000+	18	48	CCC	R	S	S	S	OOO	R	S	S	S
14	M 71	204	3,600	9	46	CCC	S	S	S	S	OOO	S	S	S	S
15	M 53	58	2,080	0	4	MCC	S	S	S	S	OOO	S	S	S	S
16	M 40	60	1,200	0	3	CMC	S	S	S	S	OOO	S	S	S	S
17	M 48	102	760	25.2	4	CMC	S	S	S	S	ScOO	S	S	S	S

Drugs: St.=Streptomycin. Sa=P.A.S. salt of isoniazid. +++=Total dosage not known in Case 3. Cultures: Cults.=Culture. C=Confluent growth. M=Moderate growth. Sc=Scanty growth. O=No growth. Sensitivities:—S=Fully sensitive. R=Fully resistant. Pr=Partially resistant.

Details of the results, together with the total amount of previous antibacterial treatment and its duration, are set out in Table II.

Resistance to Sa

In no case did full resistance to Sa develop. In Cases 1, 2, and 3 partial resistance to Sa did occur in the final sensitivity tests. In Case 1 the pre-treatment strains were fully resistant to isoniazid, but sensitive to the other drugs. At the end of treatment the strains were still sensitive to streptomycin and P.A.S., but resistant to isoniazid, and partially resistant to Sa. In this case alone was there no fall in the number of bacilli in the sputum at the end of treatment. In Cases 2 and 3 the pre-treatment strains were resistant or partially resistant to isoniazid, and partially resistant to streptomycin and to Sa. In Case 2 the strains were partially resistant to P.A.S., but in Case 3 they were sensitive. At the end of 12 weeks' treatment with Sa, sensitivities were unchanged, but there was a substantial fall in the number of bacilli excreted. Both these patients had received very large doses of P.A.S. and isoniazid during the previous three to four years.

Cases 4–10 all exhibited some degree of resistance to isoniazid at some time during the trial, and all had previously had very large doses of isoniazid and P.A.S. In none of these cases did resistance to Sa develop, and in all cases there was a marked fall in the positivity of the sputum.

Cases 11–17 never showed any resistance to P.A.S. or isoniazid in spite of the previous treatment, and resistance to Sa did not develop in any case. In six of the cases sputum conversion occurred, and in the seventh case there was a marked fall in the number of tubercle bacilli recovered from the sputum.

Drug Tolerance

Sa was well tolerated in all cases. It will be seen from Table II that most of the patients had previously taken large doses of P.A.S., isoniazid, and streptomycin. The great majority of patients expressed their appreciation of the ease with which Sa could be taken in comparison with the nausea they had associated with large doses of P.A.S.

Discussion

The most striking change following treatment with Sa is the remarkable lowering of the positivity of the sputum in a group of patients suffering from chronic bilateral pulmonary tuberculosis with cavitation. In Case 1 only was there no significant alteration in the character of the sputum. In Cases 2–11 there was a marked drop in both the volume and the bacillary content of the 24-hour samples. In Cases 12–17 sputum conversion occurred. Of the six cases which showed sputum conversion, three had had antibacterial treatment over a period of approximately four years prior to

treatment with Sa. None of these patients had previously had sputum conversion. The remaining three, although chronic bilateral cases, had previously had antibacterial treatment for a period of two to four months only. The results in these three cases suggest that Sa might be a suitable form of treatment in fresh and early cases of pulmonary tuberculosis.

In only one of the 17 cases did partial resistance to Sa develop during treatment. Two other cases, both of which had previously had very large doses of P.A.S. and isoniazid over a period of three to four years, were found to have strains of tubercle bacilli partially resistant to Sa before treatment was begun. In both these cases, however, sputum cultures became constantly less positive. In the remaining 14 cases no resistance to Sa developed.

Conclusions

This was intended as a small pilot trial to determine, firstly, that Sa was active *in vivo*, and, secondly, if drug resistance would develop quickly. Within the limits of a trial of this small size, the answer to both questions would appear to be favourable. It is hoped, therefore, on the basis of these results, to organize a larger full-scale trial of Sa. This paper is published in the hope that others actively engaged in the treatment of pulmonary tuberculosis will be encouraged to do likewise.

The cases in this trial were under the care of the following physicians at the various chest clinics and hospitals enumerated, and I would like to express my deepest thanks for their kind co-operation: Dr. J. R. Bignall, Chelsea Tuberculosis Dispensary; Dr. M. Caplin, tuberculosis officer, London Chest Hospital; Dr. Philip Ellman, East Ham Chest Clinic and Plaistow Hospital Chest Unit; Dr. L. J. Grant, St. Stephen's Hospital, Fulham; and Dr. Joseph Smart, London Chest Hospital. My thanks are also due to Messrs. Bengue and Co. Ltd. for providing the "dipasic" tablets, the P.A.S. salt of isoniazid used. I wish to express my gratitude to Miss A. M. Willings for her invaluable assistance in carrying out the laboratory side of this clinical trial.

REFERENCES

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"The specialist in abnormal psychology is not the person who is, generally speaking, best qualified to teach preventive mental health principles. The ability to grasp the implications beyond the purely technical problem is not obtained by specific knowledge of psychopathology as such, but by an understanding of normal psychological development, by the ways in which human relationships develop and in which they are expressed and realized in normal society."—*Chron. Wld Hlth Org.*, 1955, 9, 252.