FURTHER OBSERVATIONS ON THE INDIVIDUAL DIFFERENCE IN THE UPPER LIMIT OF DRUG RESISTANCE TO STREPTOMYCIN, PAS AND ISONIAZID OF TUBERCLE BACILLI OCCURRING IN SPUTA OF TUBERCULOUS PATIENTS

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The levels of drug resistance determined by the usual non-quantitative drug-resistance tests are affected by the inoculum size used for the tests. It is desirable to utilize a relatively constant size of inoculum. For this purpose, it appears to be most satisfactory to express the degree of drug resistance as the population structure of the test population of tubercle bacilli. However, because of the difficulty to describe the population structure in a simple fashion, it appeared to be practical to use the highest concentration of drug, on which small inocula consisting of 20 to 100 viable cell units can grow, as the degree of drug resistance[1]. Making data of the influence of inoculum size of the test population, drug resistances of tubercle bacilli were determined by a direct quantitative method in chronic "treatment failure" patients suffering from pulmonary tuberculosis, and it was found that the levels of drug resistance in tubercle bacilli remained within a minor variation after the long-term chemotherapy was administered repeatedly and that these levels of resistance, *i.e.*, the upper limits of drug resistance differed from patients to patients[1]. It is the purpose of this paper to present the data obtained by more systematical observations about the types of the upper limit of drug resistance in tubercle bacilli occurring in sputa of tuberculous patients.

Materials and methods

The series of patients whose sputum specimens were used in this study consisted of treatment failure patients of more than thirty years with cavitary, far-advanced pulmonary tuberculosis. They had received the long-term chemotherapy with streptomycin, PAS snd isoniazid and other drugs in various combinations before the start of this study. They showed no sputum conversion and excreted tubercle bacilli sn sputum continuously since more than several years. Tne population structure of these patients were tested by a quantitative method during two to three years course of observation. Direct drug-resistance test was performed every month or every three months.

The sputum specimens were digested by one volume of 5% potassium hydroxide to obtain three different dilutions; 10^{0} (original sputum fluid), 10^{-2} and 10^{-3}. Each of these sputum dilutions were in 0.02 ml amounts inoculated on the three series of OGAWA egg medium slants containing various concentrations of drugs by a whirl loop delivering 0.02 ml. The OGAWA egg medium consisted of the following composition: Basal solution (1% sodium glutamate and 1% KH_2PO_4), 100 ml; whole eggs, 200 ml; glycerol, 6 ml; 2% malachite green solution, 6 ml (the resulting pH 6.8). The medium was in 8 ml amounts poured into tubes, 17 to 18× 170 mm, and slanted by sterilization at 90°C for 60 minutes. Drug concentrations used were as follows: dihydrostreptomycin sulfate, 20, 200 and 2,000 μg/ml (the amounts were expressed as 10, 100 and 1,000 μg/ml, *i.e.*, one half of the original amounts after sterilization according to the common method of description in Japan, although the authors have an objectious opinion to this custum); sodium para-aminosalicylate, 1, 10, and 100 μg/ml; isoniazid, 0.1, 1, and 10 μg/ml in final concentrations. Control medium containing no drug also was added to these series. Tubercle bacilli from sputum specimens of patients who have not previously any chemotherapy as well as laboratory strains of human tubercle bacilli could not grow on the minimal concentrations of drugs described above.

The media inoculated were incubated at 37°C for six weeks. The degree of resistance was determined as the highest concentration of drug, on which more than 50% of the inoculum can grow, when the control medium showed 20 to 100 discrete colonies per tube. The degree of drug resistance determthed by the above method was named "the degree of full resistance determined by the actual count method", whereas the degree of full resistance obtained by inoculating the original sputum fluid is named "the degree of full resistance determined routine method.

Definition of the upper limit of drug resistance.

The degrees of full resistance of tubercle bacilli were plotted on charts toking the degrees of full resistance obtained by the actual count method (and also those obtained by the routine method for comparison) as the vertical axis and taking the time as the horizontal axis. When the curves of the

degree of full resistance obtained by the actual count method became horizontal, although they show one order fluctuation, over one year observation period, the degree of drug resistance, on which the curves were about horizontal, was regarded as the upper limit of drug resistance. If the curves fluctuated between two or more degrees, the degree on which the curve ran most frequently, was taken as the upper limit.

The patients received combined chemotherapy either with streptomycin, PAS and isoniazid, with isoniazid and PAS or with isoniazid and sulfisoxazole (Administration dosis: streptomycin, 1 g per day, 2 days per week; isoniazid, 0.3 to 0.4 g daily; PAS, 8 g daily; sulfisoxazole, 3 g daily). The patients always received, therefore, treatment with isoniazid with various combinations throughout the observation period of two to three years. During the observation period, neither significant improvement of clinical figures nor sputum conversion were observed in all patients.

Results and discussion

(1) Individual difference in the upper limit of drug resistance of tubercle bacilli occurring in sputa of patients.

The results obtained are shown in tables 1 and 2, and some examples of the results are shown in the previous report[1].

The upper limits of drug resistance varied from patient to patient irrespective of the fact that these patients had been administered with combinations of streptomycin, PAS and isoniazid repeatedly without any significant clinical improvement and, in addition, they had far-advanced disease, *i.e.*, similar types of the clinical figures.

(a) About one half of patients represented the upper limit of streptomycin resistance in more than 1,000 μg/ml. On the other hand, some patients showed the upper limit of streptomycin resistance in 100 μg/ml or in 10 μg/ml despite of long-term streptomycin treatment.

(b) A large number of patients (62%) showed the upper limit of PAS resistance in 10 μg/ml and the individual variation was the smallest in relation to PAS resistance among three drugs, slthough the most significant fluctuation was observed within each patient in relation to PAS resistance. In most cases the degree of full resistance to PAS by the actual count method fluctuated from time to time and, however, the upper limit of PAS resistance stayed mainly in the level of 10 μg/ml in many cases.

(c) The upper limit of isoniazid resistance varied markedly from patient to patient. A considerable number of patients showed the upper limit in 1 μg/ml and some patients showed that in 0.1 μg/ml. Only a little number of patients showed the upper limit in 10 μg/ml.

Table 1. The upper limit of drug resistance in tubercle bacilli occurring in chronic treatment failure patients

Patient Number	Upper Limit of Drug Resistance			
	Name	Streptomycin (μg/ml)	PAS (μg/ml)	Isoniazid (μg/ml)
1	M. I	100	10	0.1
2	S. I	100	10	0.1
3	M. N.	1000	10	0.1
4	S. S.	1000	10	1
5	M. F	10	10	1
6	Y. K.	10	10	0.1
7	T. I.	10	10	1
8	H. G.	10	1	0.1
9	T. S.	1000	100	1
10	S. S.	1000	1	1
11	H. H.	100	100	0.1
12	K. I.	100	10	1
13	G. K.	10	10	0.1
14	N. K.	1000	10	1
15	S. K.	10	1	0.1
16	M. M.	100	10	0.1
17	W. S.	1000	1	0.1
18	N. T.	100	100	10
19	T. S.	10	10	1
20	S. H.	1000	10	10
21	K. K.	100	100	10
22	S. S.	1000	1	1
23	T. H.	10	100	10
24	Y. F.	100	1	1
25	M. S.	100	10	1
26	M. M.	100	10	1
27	Y. O.	1000	10	0.1
28	T. T.	1000	10	1
29	Y. I	1000	10	1
30	K. N.	1000	10	0.1
31	S. K.	1000	1	0.1
32	Y. O.	1000	10	1

Table 2. Distribution of the upper limit of drug resistance in tubercle bacilli occurring thirty-two treatment-failure patients

	Upper Limit of Drug Resistance		
Streptomycin	1000 μg/ml	100 μg/ml	10 μg/ml
	14/32(44%)	10/32(31%)	8/32(25%)
PAS	100 μg/ml	10 μg/ml	1 μg/ml
	5/32(16%)	20/32(62%)	7/32(22%)
Isoniazid	10 μg/ml	1 μg/ml	0.1 μg/ml
	4/32(12%)	15/32(47%)	13/32(41%)

(2) Correlations among the upper limits of drug resistance.

Correlation between the upper limits of streptomycin resistance and PAS resistance, that between those of PAS resistance and isoniazid resistance, and that between those of streptomycin resistance and isoniazid resistante were plotted in each patient in order to examine whether a close correlation exists or not between these drugs. The results are shown in figure 1. They indicate that there is no

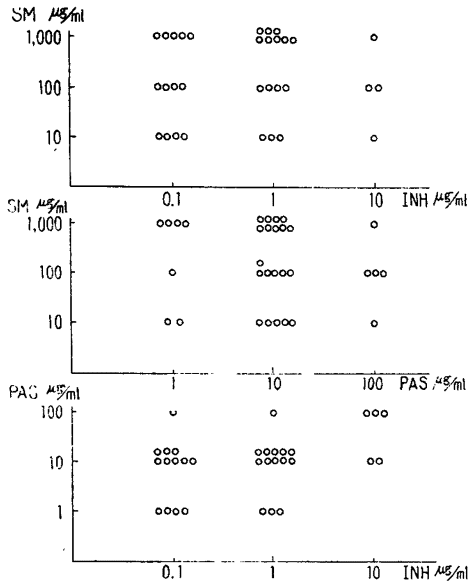

Figure 1. Relationships between the upper limits of streptomycin resistance and isoniazid resistance, between those of streptomycin resistance and PAS resistance, and between those of PAS resistance and isoniazid resistance.

correlation between the upper limits in respect to these drugs.

(3) Relationship between the upper limit of drug resistance and the clinical figure.

The patients showed no improvement by the administration of streptomycin, PAS and isoniazid made for several years. Therefore, the upper limits of drug resistance appear to indicate the limit of the chemotherapeutic effect. Among them, it appears to be remarkable that a number of patients have shown no clinical improvement by the long-term administration of isoniazid in various combinations with other drugs irrepective of the fact that these patients had bacterial populations mainly consisting of tubercle bacilli resistant to 0.1 μg/ml but susceptible to 1 μg/ml isoniazid.

It may be concluded from the data shown in this study that the effect of chemotherapeutics in a positive sense may differ from patient to patient.

In the present state of the study, the origin of the individual difference in the upper limit of drug resistance is unknown. However, in view of the recently accumulated data on the individual difference in the blood level of bacteriologically active isoniazid[2-7], it would be easily considered that, at least in respect to isoniazid resistance, the difference may be derived from this individual difference in the active level of isoniazid. In order to examine this possibility, the serum concentration of isoniazid was determined in 27 patients according to the vertical diffusion method utilizing *M. tuberculosis* H 37 Rv as the test organism[8]. The patients were administered with a single dosis of 4 mg isoniazid per kg body weight and their serum levles of isoniazid were determined 6 hours after the administration. Patients representing serum isoniazid levles less than 0.2 μg/ml, those between 0.2 and 0.8 μg/ml, and those more than 0.8 μg/ml were designated as rapid inactivators, intermediate inactivators and slow inactivators, respectively. Expecting to detect any relationship beween the upper limit of isoniazid resistance and the serum concentration of isoniazid, a number of experiments were undertaken (figure 2). From the results obtained until now, it appears still difficult to obtain any clear conclusion, since the data included only three cases of slow inactivators possibly derived from a characteristic figure of Japanese in respect to isoniazid inactivation[8,5]

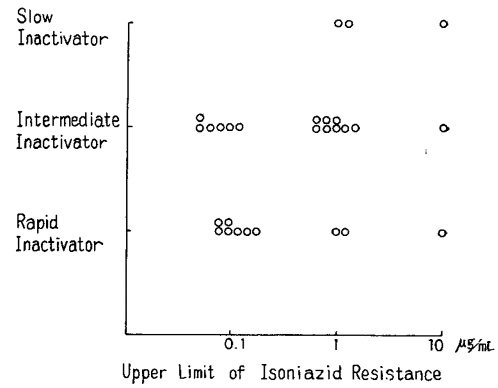

Figure 2. Relationship between the serum isoniazid concentration and the upper limit of isoniazid resistance

Summary

Population structures of tubercle bacilli were followed up in relation to streptomycin resistance, PAS resistance and isoniazid resistance during two to three year observation periods in thirty-two patients who had received chemotherapy with these drugs repeatedly and have had treatment failure cavitary pulmonary tuberculosis.

The degree of resistance was expressed as the highest concentration of drug, on which small inocula consisting of 20 to 100 viable bacilli could show more than 50% survival. The upper limit of drug resistance was defined as the average degree of full resistance from values considered to be stable over one year.

The upper limit of streptomycin resistance was more than 1,000 μg/ml in about one half of patients, and some patients showed the upper limit in 100 μg/ml and some showed that in 10 μg/ml.

The upper limit of PAS resistance was 60 μg/ml in about 60% of patients. Individual difference of the upper limit seemed to be the smallest in respect

to PAS resistance, in spite of the presence of relatively frequent fluctuation of the degree of resistance within each case.

The upper limit of isoniazid resistance varied from patient to patient. Some patients showed the upper limit in 0.1 μg/ml and some showed that in 1 μg/ml. Only a few patients showed that in 10 μg/ml.

Any positive correlation between the upper limits could not be detected in relation to these three drugs.

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