THE QUANTITATIVE ANALYSIS OF THE DRUG RESISTANCE OF *MYCOBACTERIUM TUBERCULOSIS* IN PATIENTS. REPORT III

The Analysis of PAS Resistance

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The occurrence of PAS-resistant *Mycobacterium tuberculosis* has been reported by several workers[1~3], and KARLSON, *et al.*[3] suggested that sputa of patients who received PAS may contain a heterogeneous population with respect to PAS resistance. NITTI, *et al.*[4] reported that PAS-resistant strains of *M. tuberculosis* are not stable *in vitro* as well as *in vivo* and PAS resistance is reversible by transfers of strains. OGURA[5] and ITO[6] reported similar results. TSUKAMURA[7~8] reported that the heterogeneity of population in the PAS-resistant strain of *M. tuberculosis* is not derived fom the insufficient selection of mutants but form the nature of the PAS-resistant strain of *M. tuberculosis* itself.

However, there is none of the quantitative study on the PAS resistance of bacterial bopulation occurring in sputum. The purpose of the present paper is to study the *in vivo* stability of PAS resistance and the patten of emergence of PAS resistance during the combined therapy with isoniazid and PAS (isoniazid-PAS therapy).

Materials and Methods

Specimens of sputum were treated with an equal volume of 5% potassium hydroxide for 15 minutes. The sputum hydrolyzed was diluted with saline and 100-to 10^4-dilutions were prepared. Each 0.02 ml of each dilution was inoculated on OGAWA's medium containing various concentrations of streptomycin, PAS and isoniazid. Colony count was made at 6 weeks. From these counts calculation was made of the total bacterial population and of the proportion of cells in each cultures resistant to each concentrations of the drugs. The composition of OGAWA's medium is as follows: Basal solution (1% sodium glutamate, 1%KH_2PO_4), 100 ml; eggs 200 ml; glycerol, 6 ml; 2% malachite green solution, 6 ml. Each tube contained 8 ml of medium and slanted by sterilization at 90°C for 60 minutes.

The examination was made bi-monthly on 15 patients with far advanced pulmonary tuberculosis with chronic cavities. Clinical figures were observed by content of acid-fast organisms in sputum, blood sedimentation rate, temperature, weight, and roentgenogram.

During 6 to 16 months the patients received isoniazid-PAS therapy (Isoniazid 300 mg twice or thrice weekly and PAS 8 to 10 g daily). The amount of isoniazid administered was 20 to 106 g in total and that of PAS 1,440 to 4,100 g. However, one fetal case received isoniazid 4.5 g and PAS 700 g in total.

In the present paper, the results of PAS resistance is reported. All of the patients had received the administration of PAS previously, and, only the change of bacterial population with respect to PAS resistance was observed in this report.

Results

The change of bacterial population bi-monthly observed is shown in Table 1.

Table 1 The change of bacterial population occurring in sputum bi-monthly observed (The number indicates the percentage of organisms resistant to 1, 10, 100 mcg of PAS respectively.)

Case no.	Effect of INAH-PAS-therapy	Percentage of resistant organisms per total viable units							
1	Improvement								
	1 mcg	100	41	100	87	100	100	100	
	10	100	8	67	87	83	100	100	
	100	50	0	0	0	0	1	0	
2	No change								
	1 mcg	52	52	100	74	74	100	8	100
	10	52	9	0	77	0	0	2	0
	100	0.01	0	0	0	0	0	0	0
4	Improvement								
	1 mcg	100	73	100					
	10	100	21	100					
	100	0	0	0					
5	Deterioration								
	1 mcg	100	56	100	33	58	100	68	100
	10	2	0.5	88	36	58	100	57	100
	100	0.1	0	34	22	70	100	13	100
6	Deterioration (fetal)								
	1 mcg	36	43	75					
	10	15	45	50					
	100	0	0	0					
7	No change								
	1 mcg	100	100	80					
	10	100	100	80					
	100	0	0	0					
8	Deterioration								
	1 mcg	55	42	100	15	11	100		
	10	24	32	30	0	11	100		
	100	0.01	0	0	0	0	81		

9	No change									
	1 mcg	100	100	100	49	100	80			
	10	76	100	100	36	100	69			
	100	76	18	0	35	35	20			
10	No change									
	1 mcg	100	100	18	12	69				
	10	36	100	0	3	36				
	100	0	0	0	0	0				
11	Impro ment									
	1 mcg	75	64	100	100	100	100	31	80	67
	10	0.3	3	33	1	1.4	7	10	23	42
	100	0	0.02	1.3	0.2	0.9	0.3	13	8	15
12	No change									
	1 mcg	100	100	100	100	40				
	10	51	100	100	100	40				
	100	18	100	76	65	0.04				
13	No change									
	1 mcg	0.25	70	100	64	5	50	66	69	91
	10	0	0	100	8	0.1	0.1	19	4	18
	100	0	0	0	0	0.01	0	0	0	0
14	No change									
	1 mcg	43	14	81	100	100	81	100	100	
	10	26	1	60	2.4	3	0.8	0	0.06	
	100	0	0	0	0.07	0	0.01	0	0	
15	Improvement									
	1 mcg	70	50	0	0	2	10	35		
	10	0	0	0	0	1	0	3		
	100	0	0	0	0	1	0	3		
16	Improvement									
	1 mcg	63	100	100	59	58				
	10	19	56	67	0.7	0				
	100	0	0	0	0	0				

(1) The pattern of emergence of PAS resistance during isoniazid-PAS therapy.

All of the patients had more or less bacterial population containing above 50% of organisms resistant to 1 mcg of PAS. Therefore, the change of bacterial population was observed with respect to the percentage of organisms resistant to 10 mcg of PAS (10 mcg-R) and to 100 mcg of PAS (100 mcg-R).

With respect to the percentage of 10 mcg-R, the pattern can be divided into the following 5 groups.

(a) Increase of PAS resistance *i. e.*, increase of the percentage of 10 mcg-R from 0% to a considerable rate·····4 cases.

Case 5 (from 2% to 100%); case 11 (from 0.3% to 42%); case 13 (from 0% to 18%); case 15(from 0% to 3%).

(b) Reduction of PAS resistance *i. e.*, reduction of the percentage of 10 mcg-R from a considerable rate to 0%)·····3 cases.

Case 1 (from 52% to 0%); case 14 (from 26% to 0%); Case 16 (from 19~67% to 0%).

(c) Maintainance of PAS resistance with remission. A considerable rate of 10 mcg-R was observed at the beginning of isoniazid-PAS therapy, and, during the therapy, the reduction of the peprcentage until 0% occurred and then again an increase of the percentage until a considerable rate····· 1 case.

Case 10 (from 36% to 0% and from 0% to 36%).

(d) Maintainance of PAS resistance without remission·····7 cases.

Cases 1, 4, 6, 7, 8, 9, 12.

(e) No. PAS resistance *i. e.*, no occurrence of 10 mcg-R throughout the therapy·····No case.

It appears to be important that a significant reduction of PAS resistance occurred even during the isoniazid-PAS therapy. In the cases that belong to group (b), bacterial population had contained a considerable number of 10 mcg-R at the beginning of the therapy and the 10 mcg-R disappeared during the therapy. The pattern of PAS resistance as shown in groups (b) and (c) indicates that PAS resistance is very unstable.

With respect to 100 mcg-R, the pattern can be divided into 5 groups as mentioned above.

(a) Increase of PAS resistance·····3 cases.

Case 5 (from 0.1% to 100%); case 8 (from 0% to 81%); case 11 (from 0% to 15%).

(b) Reduction of PAS resistance·····2 cases.

Case 1 (from 50% to 0%); case 12 (from 18~100% to 0%).

(c) Maintainance of PAS resistance with remission·····1 case.

Case 9 (from 76% to 0% and from 0% to 20%).

(d) Maintainance of PAS resistance without remission·····No case.

(e) No PAS resistance·····9 cases.

Cases 3, 4, 6, 7, 10, 13, 14, 15, 16.

With respect to mcg-R also, it is remarkable that reduction and remission of PAS resistance have occurred even during administration of PAS.

(2) The variation of the percentage of PAS-resistant organisms occurring in sputum *i. e.*, variation of bacterial population with respect to PAS resistance.

(i) Variation of the percentage of 1 mcg-R.

All of the patients had more or less bacterial population containing above 50% of 1 mcg-R. Among the patients, 10 cases (10/15=66.6%) showed significant variations of the percentage *i. e.*, variations of above 50% in number of the percentage.

(ii) Variation of the percentage of 10 mcg-R.

13 Patients had more or less bacterial population containing above 50% of 10 mcg-R. Among the patients, 11 cases (11/13=84.7%) showed significant variations of the percentage *i. e.*, variations of above 50% in number of the percentage.

(iii) Variations of the percentage of 100 mcg-R.

5 Patients had more or less bacterial population containing above 50% of 100 mcg-R. All of these patients showed significant variations in their bacterial populations, *i. e.*, variations above 50% in number of the percentage.

The percentage of PAS-resistant organisms varied most significantly even in the same patients. The variation of bacterial population seems to be most significant with respect to 100 mcg-R, and moderately significant with respect to 10 mcg-R, and less significant with respect to 1 mcg-R.

(3) The relationship between the occurrence of organisms resistant to 100 mcg of PAS and the therapeutic effect of isoniazid-PAS therapy.

During the isoniazid-PAS therapy, the occurrence of 100 mcg-R was observed in 6 cases (cases 1, 5, 8, 9, 11, 12) and not observed in 9 cases (cases 3, 4, 6, 7, 10, 13, 14, 15, 16). Accordingly,

(a) Cases whose bacterial bopulation contained 100 mcg-R.

Improvement 2 ; No change 2 ; Deterioration 2.

(b) Cases whose bacterial population 'cotnained no 100 mcg-R.

Improvement 3 ; No change 5 ; Deterioration 1.

If one supposed improvement as +1, no change as 0, and drterioration as −1, the therapeutic effect would be expressed as the arithmetic mean of these numbers. Accordingly,

The effect on (a) is 0 and that on (b) +0.222.

There is no significant difference between these effects.

When the effect was compared in cases whose population contained above 50% of 100 mcg-R and cases whose population below 50%.

(a) Cases whose bacterial population contained above 50% of 100 mcg-R.

Improvement 1 ; No change 2 ; Deterioration 2.

If calculated as mentioned above, the effect of the isoniazid-PAS therapy on (a) is −0.20.

(b) Cases whose bacterial population contained below 50% of 100 mcg-R.

Improvement 4; No change 5; Deterioration 1.

The effect is +0.30.

Therefore, it appears that group (a) is better than group (b). However, statistical calculation indicated that there is no significant difference between these effects in these two groups.

(4) The relationship between the occurrence of osganisms resistant to 10 mcg of PAS and the therapeutic effect of isoniazid-PAS therapy.

(a) Cases whose bacterial population contained above 50% of 10 mcg-R in the latest 6 months of the therapy.

9 Patients belonged to this groups (cases 1, 3, 4, 5, 6, 7, 8, 9, 12).

Improvement 2; No change 4; Deterioration 3.

The effect of the therapy on (a) is −0.111.

(b) Cases whose bacterial population contained below 50% of 10 mcg-R in the latest 6 months of the therapy.

6 Patients belonged to this group (cases 10, 11, 13, 14, 15, 16).

Improvement 3 ; No change 3; Deterioration 0.

The effect of the therapy on (b) is+0.500.

It appeared that there was a significant difference between the effect of these two groups. However, statistical calculation indicated that there is no significant difference.

(5) The relationship between the pattern of emergence of PAS resistance and the therapeutic effect of isoniazid-PAS therapy.

The patients could be divided into the following two groups : (a) the maintainance of PAS resistance without remission and (b) the others.

With respect to 100 mcg-R, there is no case of manintainance of resistance. Therefore, observations were made only with respect to 10 mcg-R.

(a) Cases of the maintainance of PAS resistance.
Improvement 2; No change 3; Deterioration 2.
The effect on (a) is 0.

(b) The other cases.
Improvement 3 ; No change 4 ; Deterioration 1.
The effect on (b) is+0.25.

The tendence of improvement in the group (a) wae seen. However, the difference is statistically not significant.

Discussion

In view of the results, it appears of very characteristic that a reduction of the percentage of PAS-resistant organisms occurs even during the administration of PAS, and that a marked variation of the percentage also occurs. Similar phenomena have been shown with respect to isoniazid resistance [9~11]. However, these strains do not show such marked variation of resistance with respect to streptomycin resistance as shown in PAS resistance (the second report) Considering these facts, it appears that the variation and the reduction of bacterial population with respect to PAS resistance are derived from a character of PAS-resistant strains than a reflection of organisms derived from various sites. TSUKAMURA has reported previously that a PAS-resistant strain isolated in the laboratory has a very heterogeneous composition of population and the heterogeneity is not derived from unsufficient selection of the strain but one of the nature of the strain. It appears therefore reasonable that the variation and the reduction of the percentage of PAS-resistant organisms are derived from the heterogeneity of bacterial population as the nature of the PAS-resistant strain.

In the present study, a tendency of deterioration was seen in patients with the maintainance of PAS resistance and with high percentages of PAS-resistant organisms in their bacterial population. However, none of the statitically significant relationship could not been detected between the percentage

of PAS-resistant orgaisms occurring in sputum and the therapeutic effect of isoniazid-PAS therapy.

It may be probable that the effect is concerned rather with isoniazid resistance or streptomycin resistance that with PAS resistance.

Summary

The quantitative analysis of the bacterial population occurring in sputum was made with respect to streptomycin resistance, isoniazid resistance and PAS resistance in 15 patients with far advanced pulmonary tuberculosis. In the present paper, the results have been presented as regards PAS resistnace.

The analysis was bi-monthly made for 6 to 16 months and the pattern of emergence of PAS resistance was observed during isoniazid-PAS therapy.

Even during the administration of PAS, a marked reduction and a marked variation of the percentage of PAS-resistant organisms per total viable units occurred.

A tendency of deterioration was observed in patients whose bacterial population in sputum contained a high percentage of PAS-resistant organisms (organisms resistant to 10 mcg and to 100 mcg of PAS) and a tendency of improvement in patients whose bacterial population contained a low percentage or none of PAS-resistant organisms. However, the difference of these two groups was statistically not significant. In appears that the therapeutic effect of isoniazid-PAS therapy is more closely correlated with resistance to isoniazid or streptomycin than to PAS.

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References

(1) SIEVERS, O.: Sensitivity of tubercle bacilli. Lancet, 1: 798, 1949.

(2) DELAUDE, A., KARLSON, A.G., CARR, D. T., FELDMAN W. H., & PFEUTZE, K. H.: Increase of resistance of tubercle bacilli to sodium p-aminosalicylic acid : Observations on cultures isolated from patients during chemotherapy. Proc. Staff Meet. Mayo Clin., 24 : 341, 1949.

(3) KARLSON, A. G., DELAUDE, A., CARR, D. T., PFEUTZE, K. H.. & FELDMAN, W. H. : The occurrence of tubercle bacilli resistant to p-aminosalicylic acid (PAS). Dis. of Chest, 16: 667, 1949.

(4) NITTI, V., DE FRANSISCIS, V., & CANTARELLA, R. : Sulla stabilita della PAS-resistenza *in vitro* e *in vivo*, Arch. di Tisiol., 5: 444, 1950.

(5) OGURA, S. : Clinical studies on resistance in treatment with antituberculous agents (1). Kekkaku, 30: 446, 1955.

(6) ITO, Y., KONDO, H., & MIZUNOE, K. : A study on PAS resistance. Its relation to clinical symptoms and SM-resistance, the change of resistance in the course of treatment. Kekkaku, 30 : 643, 1955.

(7) TSUKAMURA, M., & MIURA, K.: Comparison of the composition of population between the streptomycin-resistant strain, the isoniazid-resistant strain of *Mycobacterium tuberculosis* var. ***hominis***. Annual Report of the Japanese Association for Tuberculosis, 2 : 1, 1957.

(8) TSUKAMURA, M.: Properties of PAS-resistant *Mycobacterium tuberculosis*. Am. Rev. Tuberc., in press.

(9) TOMPSETT, J.D. : Quatitative observations on the pattern of emergence of resistance to isoniazid. Am. Rev. Tuberc., 70 : 91, 1954.

(10) SATO, N. : The fate of isoniazid-resistant tubercle bacilli in tuberculous patients. Kekkaku, 29 : 393, 1954.

(11) STEWART, S.M. : Varied degrees of isoniazid resistance within strains of tubercle bacilli from sputum and pulmonary cavities. Am. Rev. Tuberc., 73 : 390, 1956.