STUDIES ON THE COMBINED CHEMOTHERAPY OF STREPTOMYCIN AND ISONICOTINIC ACID HYDRAZIDE

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Chapter I Introduction

Although it is well known that streptomycin (SM) and isonicotinic acid hydrazide (INH) are for themselves the most powerful drugs for tuberculosis, the combined use of these two is rather exceptional in practice. In Japan, the commonest regimen for the chemotherapy of tuberculosis is the combination of SM and PAS, although the combined use of three drugs, SM, PAS and INH is becoming more and more popular nowadays. One of the reasons of this exceptional use of SM plus INH may be to put at least one of the most powerful drugs aside for such an urgent occasion as surgical operation. The other reason may be that PAS is considered to be most useful for preventing the development of the drug-resistant bacilli, and, therefore, the withdrawal of PAS from the combination of drugs is thought not to be rational.

However, it may be of some value to use these two powerful drugs, SM and INH, in combination in order to obtain rapid and appreciable effect for the treatment of tuberculosis without PAS, because the latter drug has rather strong side effect to the gastrointestinal and hepatic functions.

Ilavsky[1], Mackaness[2], and Hobby[3] reported that the growth of tubercle bacillus was inhibited *in vitro* in the presence of SM and INH combined even in such low doses that each drug alone did not inhibit its growth. Singh and Mitchison[4] found that the combined use of SM and INH, at the growth-inhibiting concentration of each drug, acted tuberculocidally *in vitro*. On the other hand, Ebina[5] said that the effect of SM, when used in combination with INH, became obscure by the effect of INH, and the additive effect of the two drugs was not determined. However, as for the prevention of the development of drug-resistant bacilli, Ebina as well as Ilavsky admitted the apparent preventing action of these two drugs.

Piechand et al[6] examined the effect of combined use of SM and INH on ginea pig tuberculosis, and found no significant difference between the effect of the combination and that of single drug used separately. Freerksen[7] reached the similar conclusion observing the survival time of rabbits infected with virulent tubercle bacilli. Gernez-Rieux[8], on the other hand, reported the synergistic effect of the combined chemotherapy with SM and INH on rabbit tuberculosis.

Clinically, a detailed statistics was presented by the Veterans Administration[9], that SM-INH therapy, in the minimal and moderately advanced cases, showed excellent results, but a report from Ryo-Ken[10] indicated that this therapy was less effective than the IHN-PAS therapy.

Thus, experimental and clinical results hitherto obtained seem to be contradictory. The present writer carried out some experiments on the effect of combined use of SM and INH on mouse tuberculosis, especially to see whether or not the effect of the combination is significantly better than that of single drug used separately. In addition, clinical results of 223 patients treated with combination of SM and INH will be reported in comparison with the results of the combination of SM, INH and PAS.

Chapter II Animal experiments

a) The effect of the combined use of SM and INH on the survival time of mice.

(1) Materials and methods

Seventy mice of dd strain, each weighing ca. 20 g, were used. They were divided into seven groups and all were inoculated into the tail vein with 0.5 mg (wet weight) saline suspension of two weeks old human tubercle bacilli of Kurono strain (strongly virulent to mice) grown on glycerol broth. From the next day of inoculation each group was treated as follows:

Group I: SM 20 γ/g body weight (daily)
II: INH 1 γ/g (daily)
III: SM 20 γ/g + INH 1 γ/g (daily)
IV: SM 10 γ/g (daily)
V: INH 0.5 γ/g (daily)
VI: SM 10 γ/g + INH 0.5 γ/g (daily)
VII: non-treated control

Treatments were continued until a half of mice died in each group. SM and INH were disolved with distilled water so as to make each dose described above was contained in 0.2 ml of the solution. SM was injected subcutaneously into the back of mice and INH was administered *per os* using a metal catheter.

(2) Results

Survival rates and days of each group were shown in Fig. 1. Average survival day was: 1st group 18.7, 2nd group 20, 3rd group 26.5, 4th group 17.6, 5th group 17.5, 6th group 19.3 and 7th group (control) 15.5. It seems that the combination of 20 γ/g SM plus 1 γ/g INH (3rd group) showed an apparently better effect on the survival time of mice than any regimen of single chemotherapy, but the combination of 10 γ/g SM plus 0.5 γ/g INH (6th group) had no such signifficant effectiveness.

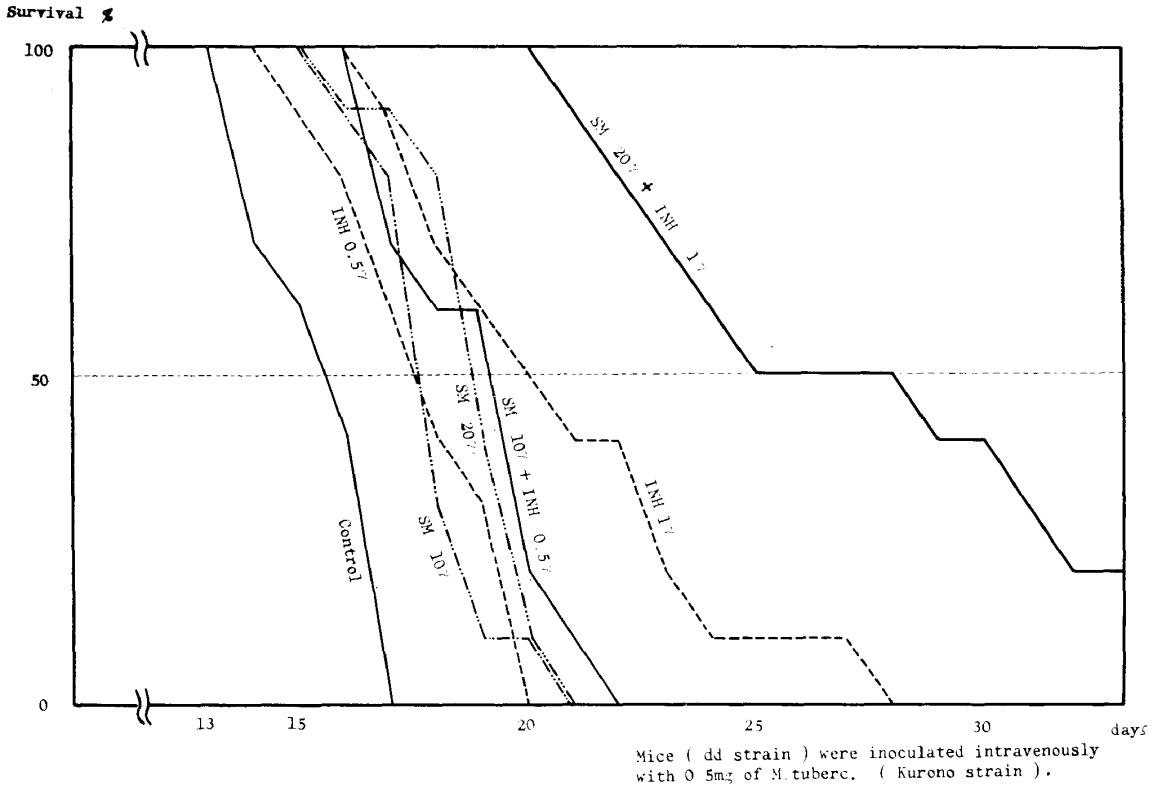

Fig. 1. Survival time of mice.

b) Evaluation of the combined chemotherapy of SM and INH by means of the quantitative cultivation of tubercle bacilli from organs.

(1) Materials and methods

Six groups of mice, each consisting of ten, were inoculated intravenously with tubercle bacilli (Kurono strain), and treated from the next day of inoculation. The doses of bacilli and drugs were as follows:

Group I: Inoculum size 0.001 mg, SM 20 γ/g body weight
II: " 0.001 mg, INH 1 γ/g
III: " 0.001 mg, SM 20 γ/g + INH 1 γ/g

IV : Inoculum size 0.01 mg, SM 20 γ/g
V : " 0.01 mg, INH 1 γ/g
VI : " 0.01 mg, SM 20 γ/g + INH 1 γ/g

Three mice from each group were sacrificed 3, 6 and 9 weeks after the onset of treatment. Quantitative cultivation of bacilli from the lung, liver and spleen was carried out as described below, and the macroscopic changes of each organ were noted.

Quantitative cultivation : A piece of organ was homogenated, and 10, 100, and 1000 fold dilutions of original homogenates were made with 6% NaOH solution. 0.1 ml of these homogenates were inoculated on the surface of 3% Ogawa's solid egg media. After 4 weeks' cultivation at 37°C, the number of colonies was counted. The estimated viable count of each organ was indicated as the number per 1 mg of the organ.

Using the cultures obtained from the lung homogenates, drug-resistance was

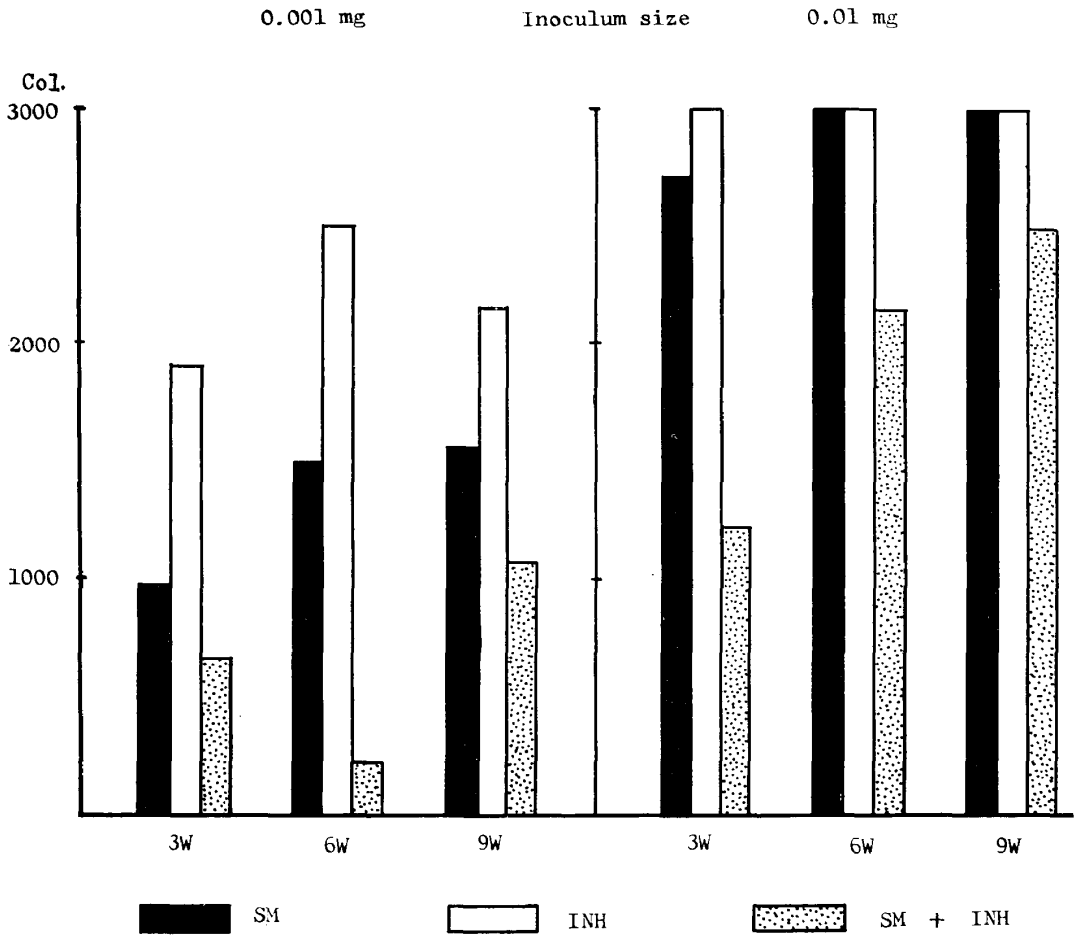

Fig. 2. Results of quantitative cultivation (Lung).

tested using the Ogawa's media containing 1, 10 and 100 γ/ml of SM or 0.1, 1 and 10 γ/ml of INH.

(2) Results

Fig. 2, 3 and 4 show the number of colonies obtained from the lung, liver and spleen in histogram, respectively.

From these results it seems that the combined use of SM and INH reduced the number of viable bacilli in organs more markedly than the single chemotherapy.

Table 1 shows the results of gross findings of organs.

From the result it may be easily known that the gross pathological changes were reduced more markedly by the combined therapy than by the single drug treatment.

Results of the tests of drug-resistance of bacilli were shown in Table 2. As for INH, no increase of resistance was seen in all bacilli tested. In the single SM treatment incomplete resistance to 10 γ/ml SM was seen in both groups of 0.001 mg and 0.01 mg.

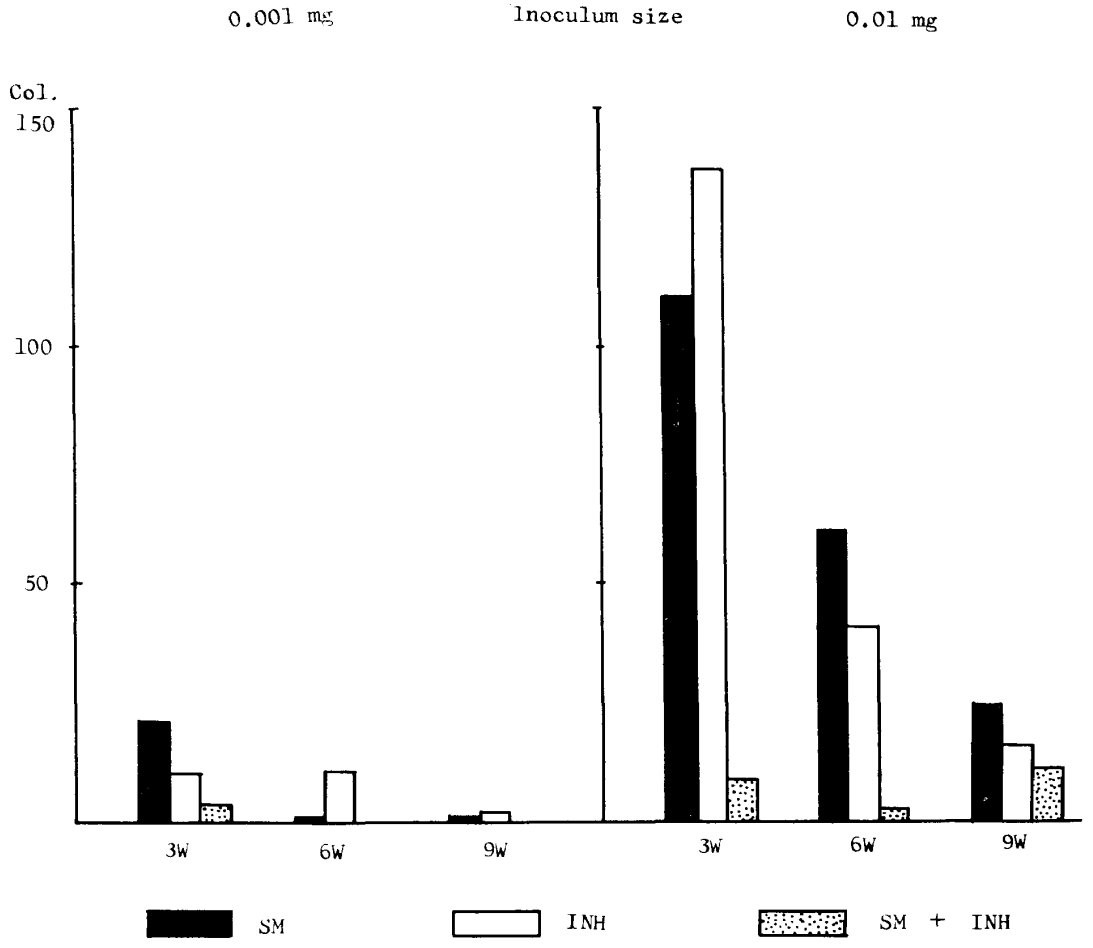

Fig. 3. Results of quantitative cultivation (Liver).

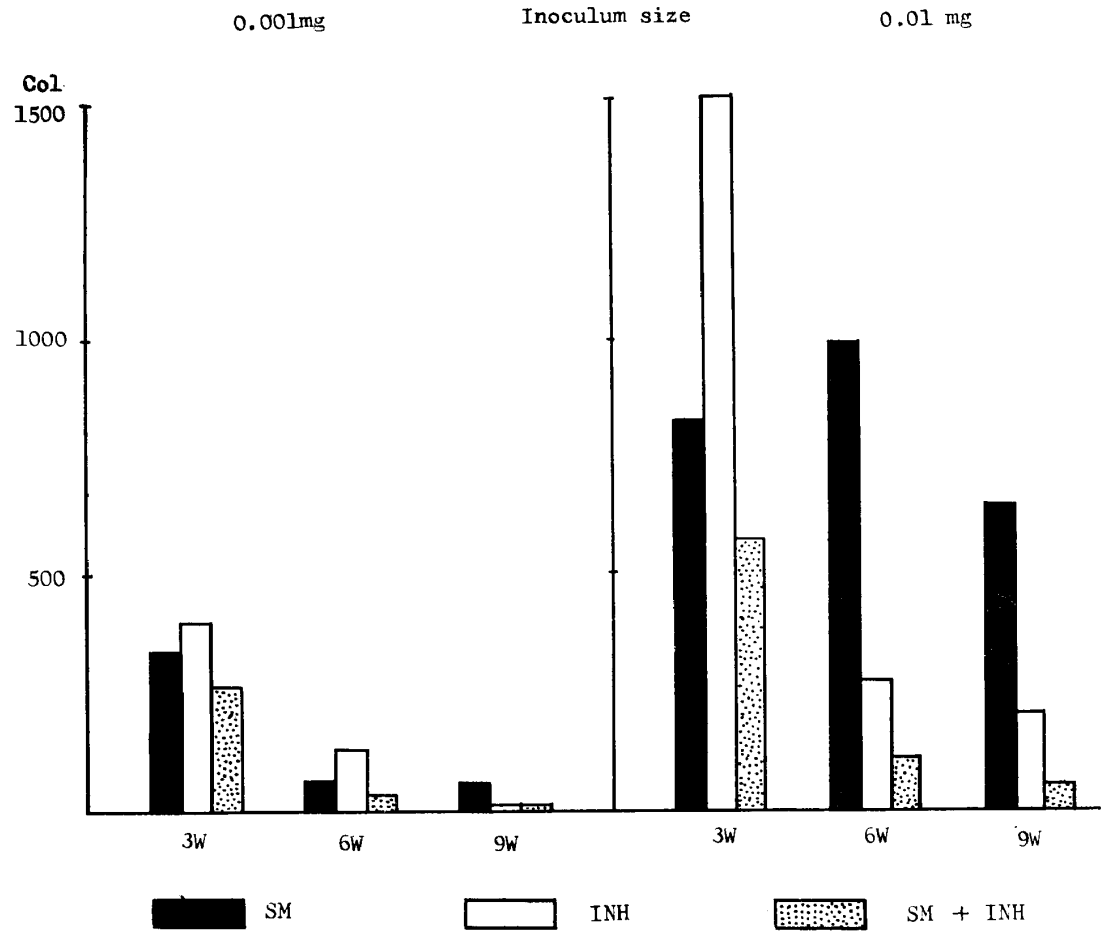

Fig. 4. Results of quantitative cultivation (Spleen).

Table 1. Results of macroscopic findings of the lung of mice.

		Inoculum size 0.001 mg	Inoculum size 0.01 mg
	Treatment	Changes	Changes
3 weeks	SM	++	++
	INH	++	++
	SM+INH	+	+
6 weeks	SM	++	+++
	INH	++	+++
	SM+INH	+	+
9 weeks	SM	+++	+++
	INH	+++	+++
	SM+INH	+	++

Note: +···a few disseminated tubercles.
++···numerous disseminated tubercles.
+++···confluent tubercles and large caseous masses.

Table 2. Results of the drug-resistance test of the bacilli obtained from lungs of mice treated with drugs.

Inoculum size	Treatment	3 weeks	6 weeks	9 weeks
0.001 mg	SM	Sensitive	Sensitive	Incomplete resistance to 10 γ/ml SM in 1 of 3 animals
	INH	Sensitive	Sensitive	Sensitive
	SM+INH	Sensitive	Sensitive	Sensitive
0.01 mg	SM	Sensitive	Sensitive	Incomplete resistance to 10 γ/ml SM in 1 of 3 animals
	INH	Sensitive	Sensitive	Sensitive
	SM+INH	Sensitive	Sensitive	Sensitive

From the experimental results described in this chapter, it may be concluded that SM and INH, when used in combination, can act more effectively on tuberculosis induced in dd strain mice than they do when used separately.

Chapter III Clinical observations

Considering the experimental results described above, it was inferred that the combined use of SM and INH might be useful for patients, especially when the side effect of PAS was expected. Clinical observations using this combination of drugs were performed in comparison with the other combined therapy which consisted of three drugs including PAS.

410 patients were treated with the following three types of chemotherapy for 6–20 months.

I) SM-INH therapy (223 cases): SM 1g twice a week, INH 0.6 mg/kg daily, but in some cases of exudative form, SM 1g daily during the first 7–10 days, and thereafter twice a week.

II) Three drugs therapy (134 cases): SM 1g twice a week, INH 0.6 mg/kg and PAS 10g daily.

III) Three drugs therapy (53 cases): SM 1g and INH 0.6 mg/kg twice a week and PAS 10g daily.

The types of the disease classified by the scheme of the National Tuberculosis Association (U. S. A.) at the onset of the treatment were shown in Table 3.

Table 3. X-ray findings before treatment (N.T.A. classification).

	SM+INH	SM+PAS+INH		
		INH daily	INH intermittent	Total
Minimal	27 (12.1%)	67 (50.0%)	22 (41.5%)	89 (47.6%)
Moderately advanced	165 (74.0%)	59 (44.0%)	24 (45.3%)	83 (44.4%)
Far advanced	31 (13.9%)	8 (6.0%)	7 (13.2%)	15 (8.0%)
Total	223	134	53	187

Table 4. Body weight changes of the patients.

	SM+INH	SM+PAS+INH					
		INH daily		INH intermittent		Total	
Loss of weight	4	(1.8%)	7	(5.2%)	4	(7.5%)	11	(5.9%)
Unchanged	13	(5.8%)	23	(17.1%)	10	(18.9%)	33	(17.6%)
Increased 2～ 4 kg	16		24		10		34	
4～ 6 kg	20		23		13		36	
6～ 8 kg	24		19		7		26	
8～10 kg	25	(92.4%)	14	(76.9%)	5	(73.6%)	19	(76.5%)
10～12 kg	29		8		4		12	
12～14 kg	27		8		0		8	
14 kg—	65		8		0		8	
Total	223		134		53		187	

Note: 6–20 months observation

a) Body weight

As shown in Table 4, in SM-INH therapy, the rate of increase of body weight was 92.4%, and about the two thirds of patients showed the increase over 10 kg. While in cases of three drugs therapy the rate of increase was 76.5%, and the increase of weight was under 10 kg in the majority of them. From the point of view of body weight, the SM-INH therapy was apparently better than the three drugs therapy.

b) Erythrocytes sedimentation rate (ESR)

It was found that SM-INH therapy was the best in the improvement of ESR, among three types of therapies examined.

c) Tubercle bacilli in sputa

Results were classified by the standard of Ryo-Ken (Tuberculosis Research Committee, supported by the Ministry of Health and Welfare) and were shown in Table 5. It seems that the three drugs therapy was better than the SM-INH

Table 5. Tubercle bacillus findings in sputa.

	SM+INH	SM+PAS+INH		
		INH daily	INH intermittent	Total
Converted negative	143 (81.7%)	52 (92.9%)	22 (84.6%)	74 (90.3%)
The number of the bacilli: Decreased	27 (15.4%)	1 (1.8%)	0	1 (1.2%)
The number of the bacilli: Unchanged	5 (2.9%)	2 (3.6%)	4 (15.4%)	6 (7.3%)
The number of the bacilli: Increased	0	1 (1.8%)	0	1 (1.2%)
Continuously negative	48	78	27	105
Total	223	134	53	187

therapy from the view-point of the negative conversion rate of the bacilli. The three drugs therapy of INH daily was much better than that of INH intermittently.

d) Development of drug-resistant tubercle bacilli.

In the present investigation the drug-resistance means that when the bacilli grow *in vitro* in the concentration of more than 10 γ/ml of SM or PAS, or 1 γ/ml of INH. As seen in Table 6, it may be worthy of note that the development of drug-resistance was more frequent in the SM-INH therapy than the three drugs therapy of INH daily, and the worst was the three drugs therapy of INH intermittently.

Table 6. Development of drug-resistance of the tubercle bacilli in sputa.

	SM+INH	SM+PAS+INH		
		INH daily	INH intermittent	Total
Positive culture before treatment	175	56	26	82
Failed to convert negative	32 (18.3%)	4 (7.1%)	4 (15.4%)	8 (9.8%)
Drug-resistance developed	9 {(5.1%)* (28.1%)**	0	2 {(7.7%)* (50.0%)**	2 {(2.4%)* (25.0%)**

The percentage value of the asterisk (*) of the drug-resistant group shows the rate of drug-resistance in the positive cases at the onset of the treatment, and that of the two asterisks (**) shows the rate of drug-resistance in the cases which failed to convert negative.

Table 7. X-ray findings of the chest.

	SM+INH	SM+PAS+INH		
		INH daily	INH intermittent	Total
Markedly improved	133 (59.6%)	51 (38.1%)	11 (20.8%)	62 (33.2%)
Moderately improved	53 (23.8%)	29 (21.6%)	17 (32.1%)	46 (24.6%)
Slightly improved	26 (11.6%)	35 (26.1%)	16 (30.2%)	51 (27.3%)
Unchanged	4 (1.8%)	19 (14.4%)	8 (15.1%)	27 (14.4%)
Worsened	7 (3.1%)	0	1 (1.9%)	1 (0.5%)
Total	223	134	53	187

e) Roentgenographic findings of the chest

The changes of the X-ray findings were indicated by the formula of Gaku-Ken (Tuberculosis Research Committee, supported by Japan Education Ministry). As shown in Table 7, the marked improvement was seen more frequently in the group of SM-INH than in the three drugs therapies, whereas the worsening seemed to be slightly more in the former than in the latter.

f) Cavity

The changes of the cavity in the X-ray films were classified as follows:

(i) Disappeared: Transparence of the cavity completely disappeared remaining linear or strangular or small round shadows of which diameter was the same or less than the sum of the thickness of original cavity wall, (ii) Filled-in: Transparence of the cavity completely disappeared, but the diameter of the concentrated shadow was more than the sum of the thickness of the original cavity wall, (iii) Reduced: Transparence of the cavity did not disappear, but the outer diameter of the lesion became smaller than the half of the original cavitary thickness, (iv) Unchanged: Transparence of the cavity did not disappear, and the outer diameter of the cavity was more than half of the original cavitary thickness, (v) Enlarged: Transparence did not disappear, and the cavity enlarged.

The results were shown in Table 8. The rate of disappearance of the cavity was higher in the SM-INH therapy (63.5%) than in the three drugs therapy (49.6%).

Table 8. The changes of cavity by the treatment.

	SM+INH	SM+PAS+INH		
		INH daily	INH intermittent	Total
Disappeared	66 (63.5%)	55 (51.4%)	12 (42.9%)	67 (49.6%)
Filled-in	1 (1.0%)	4 (3.7%)	1 (3.6%)	5 (3.7%)
Reduced	31 (29.8%)	25 (23.4%)	10 (35.7%)	35 (25.9%)
Unchanged	3 (2.9%)	22 (20.6%)	4 (14.3%)	26 (19.3%)
Enlarged	3 (2.9%)	1 (0.9%)	1 (3.6%)	2 (1.5%)
Total	104	107	28	135

g) Summarized clinical evaluations of the three different therapies.

Evaluations were done according to the standard formula presented by Gaku-Ken. As seen in Table 9, the SM-INH therapy was the best in the minimal and the moderately advanced cases, but in the far advanced cases the rate of worsening was higher in the SM-INH therapy than in the three drugs therapies.

h) Side reactions

A few cases of allergic or idiosyncrasic reaction to one of these drugs were seen in each group of treatment. Besides, some cases of tinnitus and bradyacusia by SM, and peripheral neuritis by INH were also seen in all groups. The most remarkable problem was the side reaction induced by PAS such as anorexia or gastrointestinal symptoms. Twenty three cases were forced to give up the use of PAS in the course of the three drugs therapy. And there were many patients who complained anorexia, though the discontinuation of the drug was not neces-

Table 9. Summarized clinical evaluation.

a) SM+INH

	Markedly improved	Moderately improved	Slightly improved	Unchanged	Worsened	Total
Minimal	16	6	5	0	0	27
Moderately advanced	92	36	34	3	0	165
Far advanced	0	6	11	7	7	31
Total	108	48	50	10	7	223

b) SM+PAS+INH (INH daily)

Minimal	21	13	17	16	0	67
Moderately advanced	20	5	20	14	0	59
Far advanced	0	0	2	6	0	8
Total	41	18	39	36	0	134

c) SM+PAS+INH (INH intermittent).

Minimal	6	5	6	5	0	22
Moderately advanced	5	4	10	5	0	24
Far advanced	0	2	1	2	2	7
Total	11	11	17	12	2	53

sary. The medication was used to stop for three days before the monthly culture of sputa. One hundred and two patients among 187 who were treated with three drugs increased their appetite in these non-medicated days, while only one case of SM-INH therapy group was so. This fact may indicate the masked side effect of PAS, i.e., the unconscious decrease of appetite during PAS administration on more than half of the patients.

Chapter IV Conclusion

The results of the animal experiments in the present investigation suggest that the combined use of SM-INH exert a more active effect on tuberculosis than used separately.

From the clinical investigation, it may be said that (1) body weight, X-ray findings and cavity were improved by SM-INH therapy much better than SM-INH-PAS therapy; and (2) from the view-points of the bacillus findings in sputa and the development of drug-resistance, SM-INH therapy was not so good as three drugs treatment in the negative conversion rate, and, therefore, more abundant appearance of the drug-resistance was noticed in the former treatment.

This also may mean that the worsening of symptoms was more apparent in far advanced cases treated with SM-INH than three drugs combined. However, as the early negative conversion can be expected in the minimal or moderately advanced cases even with the treatment of SM-INH, and also these two drugs effect favourably for the general condition of the patients as body weight, X-ray findings, appetite and so on, the SM-INH therapy may be recommended for those patients.

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