

THE ANTITUBERCULOSIS ACTIVITY OF ISONIAZID IN COMBINATION WITH SULFISOXAZOLE (GANTRISIN) II

THE EFFECT OF ISONIAZID IN COMBINATION WITH SULFISOXAZOLE ON THE EXPERIMENTAL TUBER- CULOSIS IN GUINEA PIGS AND MICE

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(Received for publication January 13, 1960)

Introduction

The satisfactory results of the investigations on the tuberculostatic activity against *M. tuberculosis* H 37 Rv of the combined use of isoniazid (INH) with sulfisoxazole (SI) *in vitro* have recently been reported by Masukazu Naitô and his associates^{1,2)}.

The purpose of this paper is to report the effect of the combined use of isoniazid with sulfisoxazole on the experimental tuberculosis in guinea pigs and mice.

In the first and second experiment, the effects of isoniazid administered in combination with sulfisoxazole on the ocular tuberculosis in guinea pigs^{3,4,5)} were investigated.

And in the third experiment, the influence of the administration of isoniazid combined with sulfisoxazole upon the survival of the tuberculous mice infected with the virulent human type tubercle bacilli (KURONO Strain⁶⁾) was observed.

Materials and Methods

(A) For the experiment I and II, the severity of the experimental ocular tuberculosis in guinea pigs was measured.

The guinea pigs, weighing approximately 500 gm and negative to tuberculin skin reaction (0.1 cc. of 1:10 diluted O.T.), were subcutaneously inoculated with 0.1 mg. (wet weight) of *Mycobacterium tuberculosis* H 37 Rv. About four weeks later, when the positive conversion of tuberculin skin reaction was certified in all guinea pigs, their anterior chambers of the right eyes were punctured and

inoculated with 0.0025 mg. (wet weight) of the same strain in order to produce the experimental ocular tuberculosis.

The development of the ocular tuberculous lesions was precisely observed by means of Hand Slit-Lamp Examination and recorded with the values of the index of lesion of each animal.

The severity of the tuberculous lesions in the anterior segment of the eyes was classified by the index of Steenken et al⁴⁾, modified in this laboratory⁷⁾ as shown in Table 1.

Table 1. Index of experimental tuberculous lesions in the anterior segments of the guinea pig's eyes.

Findings		Index of tuberculous lesions					
		I	II	III	IV	V	VI
Conjunctiva	Ciliary injection	÷	+	++	+++	+++	Perforation or phthisis bulbi
	Edema	—	—	÷	+	++	
Cornea	Curvature	—	—	—	—	+	
	Dullness	—	+	+	++	+++	
	Vascularization	—	+	+	++	+++	
Anterior Chamber	Depth	normal	various	various	various	unclear	
	Turbidity	—	+	+	++	+++	
	Puruloid substance	—	—	+	++	+++	
Iris	Irregularity	+	++	+++	+++	unclear	
	Hyperemia & swelling	+	++	+++	+++		
	Vasodilatation	—	+	++	+++		
	Tubercles	—	various	various	various		
Pupil	Form	round	various	various	various	unclear	
	Tubercles	—	various	various	various		
	Light reflex	normal	various	various	various		

When the moderately advanced tuberculous lesions were observed about seven to ten days after the intraocular inoculation, the animals were divided into several groups in accordance with the plan of experiments. Each group involved seven to ten guinea pigs and the arithmetic mean of the indices of them was considered to be the index of the group at that time.

Body weight of experimental animals was measured weekly.

The daily oral administration of the drugs started on the day of grouping the animals.

The grouping in the experiment I and II was as follows.

Experiment I-A

- Group 1 INH 30 mg/kg
- Group 2 INH 30 mg/kg + SI 150 mg/kg
- Group 3 INH 30 mg/kg + SI 300 mg/kg
- Group 4 untreated

Experiment I-B

- Group 1 INH 15 mg/kg
- Group 2 INH 15 mg/kg + SI 45 mg/kg
- Group 3 INH 15 mg/kg + SI 150 mg/kg
- Group 4 untreated

Experiment I-C

- Group 1 INH 8 mg/kg
- Group 2 INH 8 mg/kg + SI 24 mg/kg
- Group 3 INH 8 mg/kg + SI 80 mg/kg
- Group 4 untreated

Experiment II-A

- Group 1 INH 5 mg/kg
- Group 2 INH 5 mg/kg + SI 50 mg/kg
- Group 3 untreated

Experiment II-B

- Group 1 INH 1 mg/kg
- Group 2 INH 1 mg/kg + SI 10 mg/kg
- Group 3 untreated

Experiment II-C

- Group 1 INH 0.5 mg/kg
- Group 2 INH 0.5 mg/kg + SI 5 mg/kg
- Group 3 untreated

Experiment II-D

- Group 1 INH 0.25 mg/kg
- Group 2 INH 0.25 mg/kg + SI 0.25 mg/kg
- Group 3 untreated

The mean values of tuberculous indices in each experimental group was plotted successively.

(B) For the experiment III, the survival rate of tuberculous mice was counted.

The inbred female mice (dd-strain), weighing 16 to 18 gm, were used in this experiment.

The mice were inoculated intravenously with 0.5 mg. (wet. weight) of *Mycobacterium tuberculosis* var. *hominis* (KURONO strain, isolated by K. Mizunoe⁶⁾) and divided into several groups with the plan of experiments.

The administration of the drugs started on the day of the intravenous inoculation of the tubercle bacilli and was kept along until a half of the mice in any one of the treated groups were dead.

The number of dead mice was calculated daily and plotted on the graph.

The grouping of mice in this experiment was as follows.

Experiment III-A

- Group 1 INH 5 mg/kg
- Group 2 INH 5 mg/kg + SI 50 mg/kg
- Group 3 untreated, control

Experiment III-B

- Group 1 INH 0.5 mg/kg
- Group 2 INH 0.5 mg/kg + SI 5 mg/kg
- Group 3 untreated, control

Experiment III-D

- Group 1 INH 0.25 mg/kg
- Group 2 INH 0.25 mg/kg + SI 2.5 mg/kg
- Group 3 untreated, control

Results

In the experiment I, the combined effect of INH-SI on the experimental ocular tuberculosis was evaluated with various doses of INH in combination with SI at the ratio of 1:3 and 1:10, as shown in Fig. 1, 2 and 3.

The combined effect was slight in the experiment 1-C.

It was known that the large dose of INH, such as 15 mg/kg or more, was fully effective even when it was singly used.

Therefore, it was necessary as Karlson and Feldman reported⁸⁾, to limit the dose of INH when the combined effect of INH with other drug was to be discussed.

In the successive experiments, the suitable dose of INH for the exhibition of the combined effect of INH-SI was investigated.

Results of experiment II was shown in Fig. 4, 5, 6 and 7.

The combined effect of INH-SI was noted markedly in the Exp. II-B, in which 1.0 mg/kg of INH was administrated.

In other experiments, the combined effect of INH-SI was not so marked. According to the results of experiments I and II, it may be said that the combined effect of INH-SI regimen was demonstrated remarkably when the dosage of INH was relatively small and, therefore, the therapeutic effect of INH alone was minimum such as shown in the Fig. 5.

Fig. 1. Changes of indices of experimental ocular tuberculosis of guinea pigs during treatment. (INH 30 mg/kg)

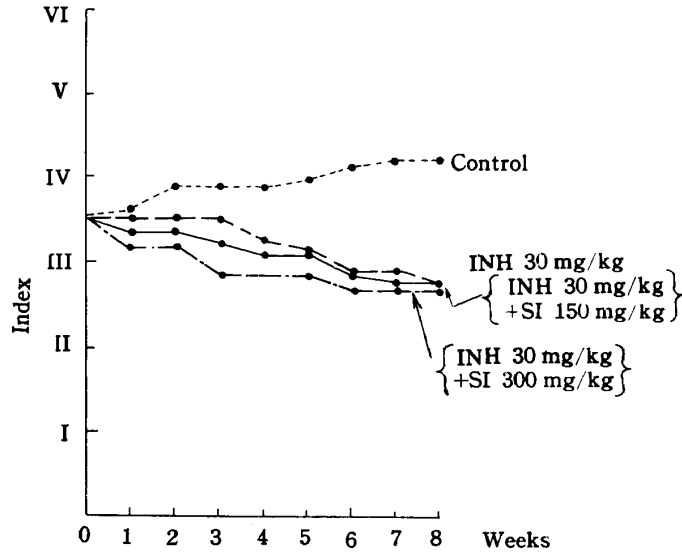


Fig. 2. Changes of indices of experimental ocular tuberculosis of guinea pigs during treatment. (INH 15 mg/kg)

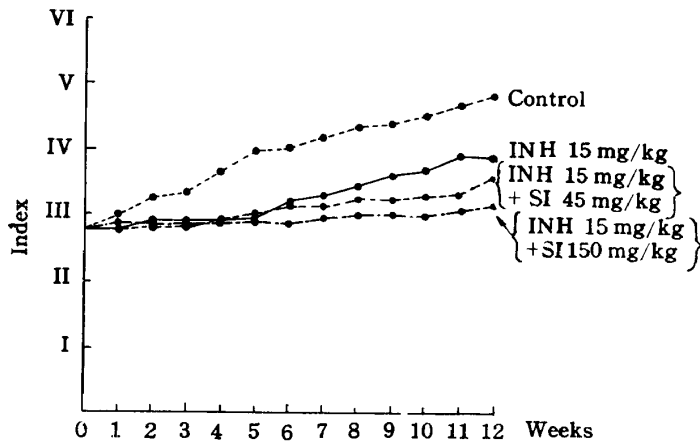


Fig. 3. Changes of indices of experimental ocular tuberculosis of guinea pigs during treatment. (INH 8 mg/kg)

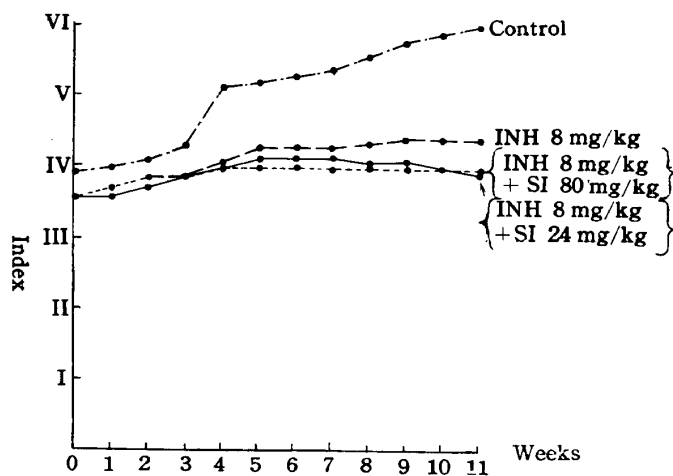


Fig. 4. Changes of indices of experimental ocular tuberculosis of guinea pigs during treatment. (INH 5 mg/kg)

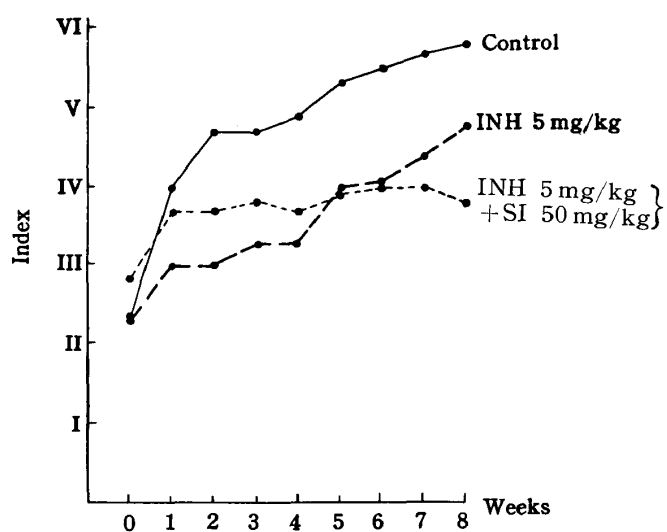


Fig. 5. Changes of indices of experimental ocular tuberculosis of guinea pigs during treatment. (INH 1 mg/kg)

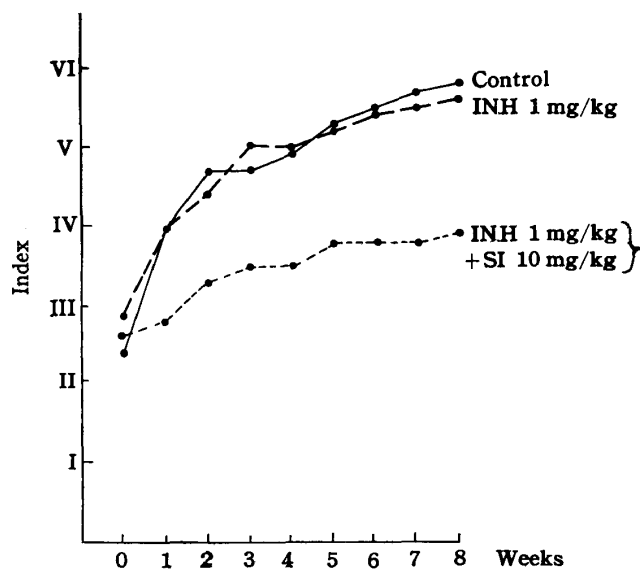


Fig. 6. Changes of indices of experimental ocular tuberculosis of guinea pigs during treatment. (INH 0.5 mg/kg)

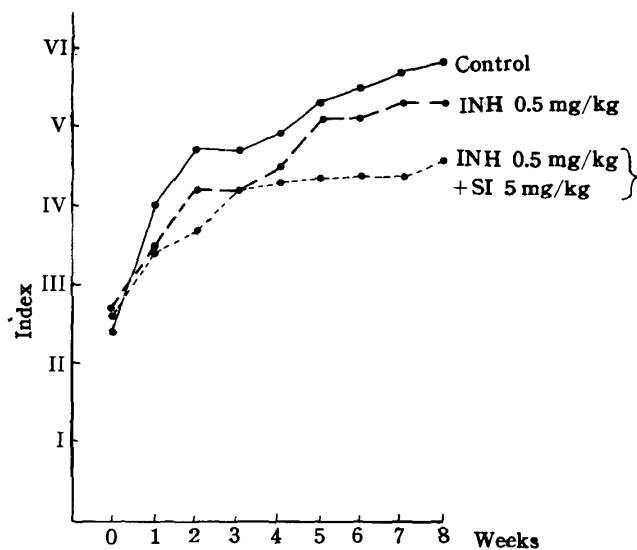


Fig. 7. Changes of indices of experimental ocular tuberculosis of guinea pigs during treatment. (INH 0.25 mg/kg)

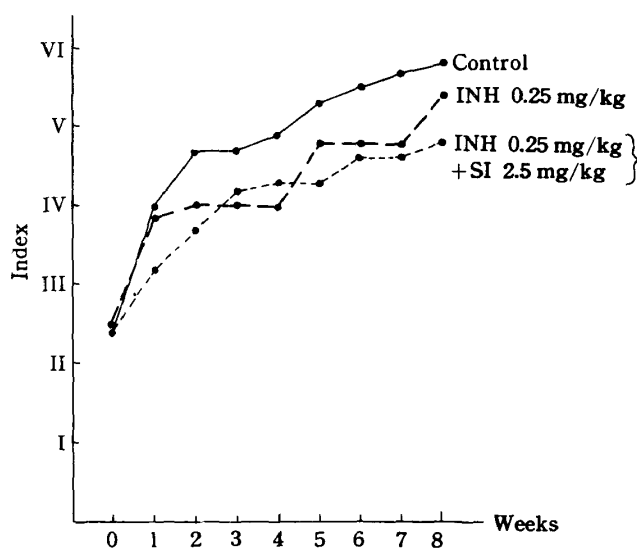
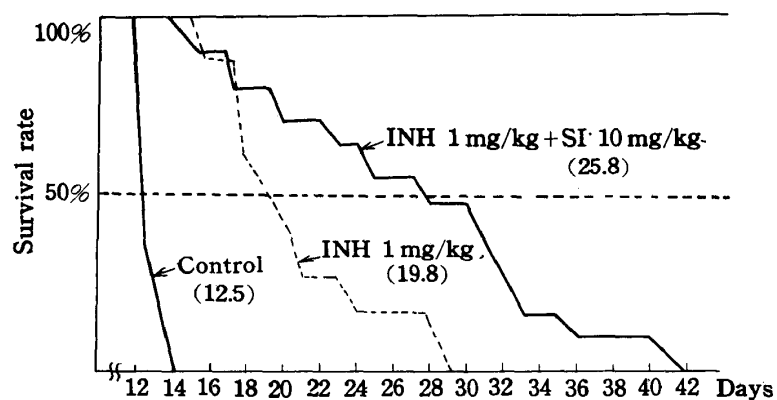
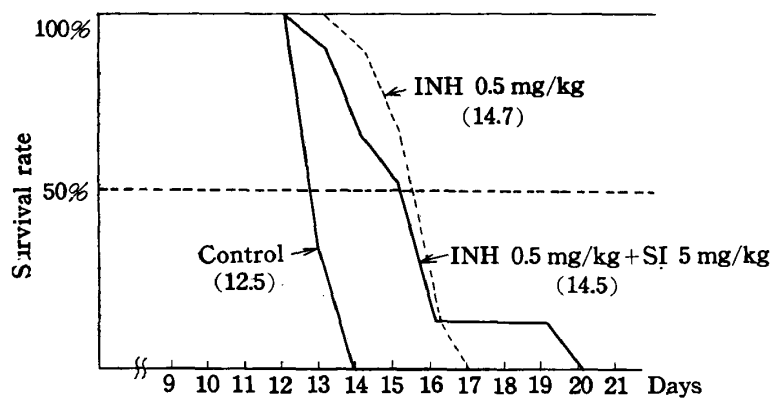


Fig. 8. The survival rate of mice infected with tubercle bacilli Kurono strain and treated with INH (1 mg/kg) or INH and SI.



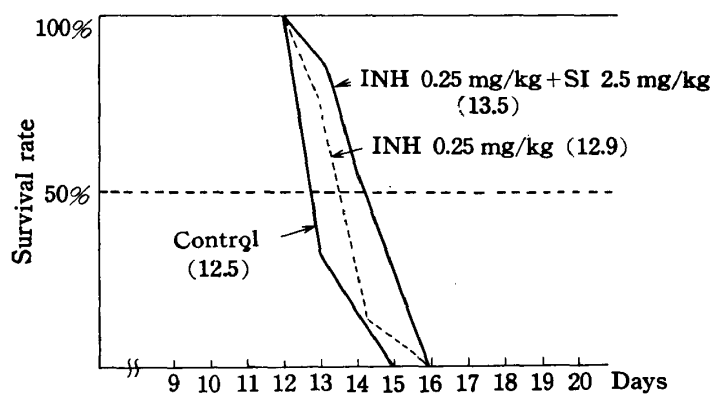
Note: The number in the parenthesis indicates the mean survival days of the group of the mice.

Fig. 9. The survival rate of mice infected with tubercle bacilli Kurono strain and treated with INH (0.5 mg/kg) or INH and SI.



Note: See footnote of Fig. 8.

Fig. 10. The survival rate of mice infected with tubercle bacilli Kurono strain and treated with INH (0.25 mg/kg) or INH and SI.



Note: See footnote of Fig. 8.

But when the dose of INH was less than 1 mg/kg of body weight of the guinea pigs as in the Exp. II-C and II-D, neither the effects of singly administered INH nor the effects of INH in combination with SI were observed.

In the Exp. III, the mice of the control group died uniformly 12 to 15 days after the intravenous inoculation of tubercle bacilli. The survival rate of each group was shown in the Fig. 8, 9 and 10.

When 5 mg/kg of INH were given daily, all the mice in the treated groups survived even at the end of the sixth week after inoculation. That is to say, 5 mg/kg of INH was fully effective when used singly and so the combined effect of INH with SI could not be demonstrated.

When 1 mg/kg of INH in combination with 10 mg/kg of SI was administered, the prolongation of the survival of the tuberculous mice was remarkable in comparison with the single administration of 1 mg/kg of INH.

When the dose of INH was 0.5 mg/kg or less, the therapeutic effect of INH administered singly or in combination with SI could not be demonstrated.

The mean survival days of each group of mice was shown in Table 2.

Table 2. The mean survival days of each group of mice.

Regimen	Mean Survival Days
INH 5 mg/kg alone	—*
INH 5 mg/kg + SI 50 mg/kg	—*
INH 1 mg/kg alone	19.8
INH 1 mg/kg + SI 10 mg/kg	25.8
INH 0.5 mg/kg alone	14.7
INH 0.5 mg/kg + SI 5 mg/kg	14.6
INH 0.25 mg/kg alone	12.9
INH 0.25 mg/kg + SI 2.5 mg/kg	13.5
Control (untreated)	12.5

* Uncalculable because none of the mice in the group died.

Discussion

It has been known that the dose of inoculum and the pH of culture media is responsible for the maximal bacteriostatic activities of some drugs *in vitro*. Similarly, it was considered in the experimental tuberculosis that the dose of inoculum and the dose of drugs were principally responsible for the maximal therapeutic effects.

Karlson and Feldman⁸⁾ stated that it was important to determine the sub-effective dose of isoniazid in order to find out the effect of other antituberculous drugs in combination with isoniazid and that the daily treatment with 0.5 mg/kg

of isoniazid was subeffective for the mature male guinea pigs, weighing approximately 800 mg, inoculated subcutaneously over the sternum with 0.1 mg. of virulent tubercle bacilli, H 37 Rv, and reported that for guinea pigs weighing approximately 800 gm, a daily single dose of isoniazid of 0.25 mg. or 0.5 mg. should be used as the subeffective dose in tests of the value of other drugs in combination with isoniazid.

The authors evaluated the effects of isoniazid in combination with sulfisoxazole in the experimental tuberculosis of guinea pigs and mice, and point out that to demonstrate the combined effect of isoniazid with sulfisoxazole, the dose of isoniazid should be small enough so that singly administered isoniazid was not completely effective.

Summary

The combined effect of isoniazid with sulfisoxazole was evaluated *in vivo* experiments. In the process of the experimental tuberculosis in the anterior segment of guinea pigs' eye and in the observation of the survival time of tuberculous mice, the combined effect of isoniazid with sulfisoxazole was clearly demonstrated.

The dosage of isoniazid to demonstrate its combined effect with sulfisoxazole was also experimentally studied.

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