Neo-nocardin, a New Antibiotic Produced by a New Species of Nocardia

II. The effect of neo-nocardin on the experimental tuberculosis in mice

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(Received in Jan. 1, 1953)

Introduction

The rapid and accurate estimation of the degree of antituberculous activity *in vivo* of synthetic and organic compounds is of considerable practical importance.

The promising results achieved in recent years with the newer chemotherapeutic agents and antibiotics, such as PAS, TB-1, and streptomycin, have made it desirable or even necessary to test objects other than the classical guinea pig for the purpose of saving time and material. Mouse tuberculosis thus came into use as the standardized test for antituberculous activity of drugs.

Many American investigators have developed methods for testing antituberculous activity in mice (1), (2), (3), (4), (5), (6).

In our conntry Iwasaki and Ogawa (7) and Ogawa and his associates (8) have also developed a new method of mice-test estimating quantitatively the tubercle bacilli contained in the organs of treated and untreated animals. They have, however, not regarded the mice-test as the standard test but as the screening test.

In the author's opinion mice-test can also be used as the standard test by itself. We are not searching for agents active against guinea pig or

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mouse tuberculosis but for the agents active against human tuberculosis. Neither the guinea pig tuberculosis nor the mouse tuberculosis are the pattern of human tuberculosis. As Mckee et al (3) said there is no evidence that the results of chemotherapy in tuberculosis of the guinea pig are a more certain guide to the usefulness of a given compound in man than the results obtained in mice. And there is also no evidence if a given compound is effective against guinea pig or mouse tuberculosis it might also be effective against human tuberculosis. From this point of view all attempts at chemotherapy in experimental tuberculosis are no more than screening tests which are followed by the chemotherapeutic evaluation in human tuberculosis. A consideration of this point of view has led me to use mice to determine the *in vivo* antituberculous activity of the crude hydrochloride extracted from the fermented broth of *Nocardia kuroishi* A. 422 (neonocardin) (9).

Materials and methods

Strain of tubercle bacillus: Frankfurt strain of Mycobacterium tuberculosis var. hominis was employed as the test organism. This organism was grown on egg medium for about 3 to 4 weeks and tne saline suspensions for inoculation into mice were made in the usual manner.

Chemotherapeutic experiment: Nine mice weighing 15 to 20 Gm were divided into three groups. Group 1— neo-nocardin, group 2— streptomycin, group 3— control. Administration of the antibiotics was by intramuscular injection with a daily dose of 1.0 mg.

The infecting does of 0.1 mg of tubercle bacillus in a volume of 0.1 ml of saline suspension was administered intravenously.

All the treated and control animals were sacrificed at the next day the treatment was terminated. At the time of necropsy an estimation was made of the amount of gross tuberculosis present in lungs, liver and spleen. The quantitative estimation of living tubercle bacilli contained in lungs, liver and spleen was made by the Ogawa's culture method (10).

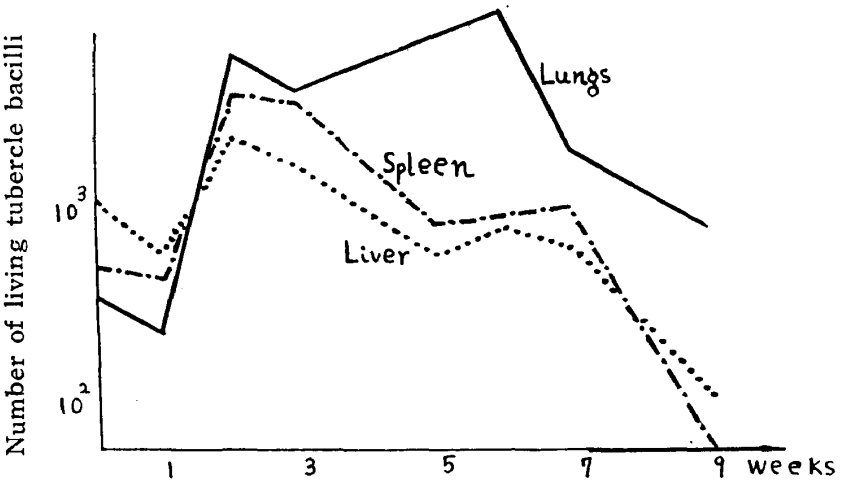
Experiment 1

Changes of number of tuberele bacilli in mice during infection.

The number of living tubercle bacili in the organs of mice during infection was investigated by the quantitative culture method (10). Figure 1 shows one of the results obtained from three separate experimehts. From the figure it may be concluded that (1) at the next day of inoculation the number of bacilli contained in liver and spleen is greater that the one contained in lungs, (2) the bacilli have a tendency to decrease in number for one or two

weeks following inoculation and (3) then begins to increase in number and the degree of increase is more remarkable in lungs than in spleen and liver.

Fig. 1. Number of living tubercle bacilli in the organs of mice during infection



Time after infection
ordinate represents the next day after infection

From the above-mentioned results it is a reasonable presumption that the development of tuberculous infection in mice can be divided into these successive stages: the first stage is from the day of inoculation to 1 to 2 weeks after inoculation, the second stage is from 1 to 2 weeks after inoculation to the time at which the number of bacilli attains its maximum and third stage is from that time onward.

It may be reasonable to think that the relation between the bacilli and the host may be different in each stage above-mentioned and the reaction to the antituberculous agents may also be different in each stage. In this reason the author treated the infected mice with neo-nocardin according to the stage separately and compared its effect with that of streptomycin.

Experiment 2

Effect of neo-nocardin upon the experimental tuberculosis in mice.

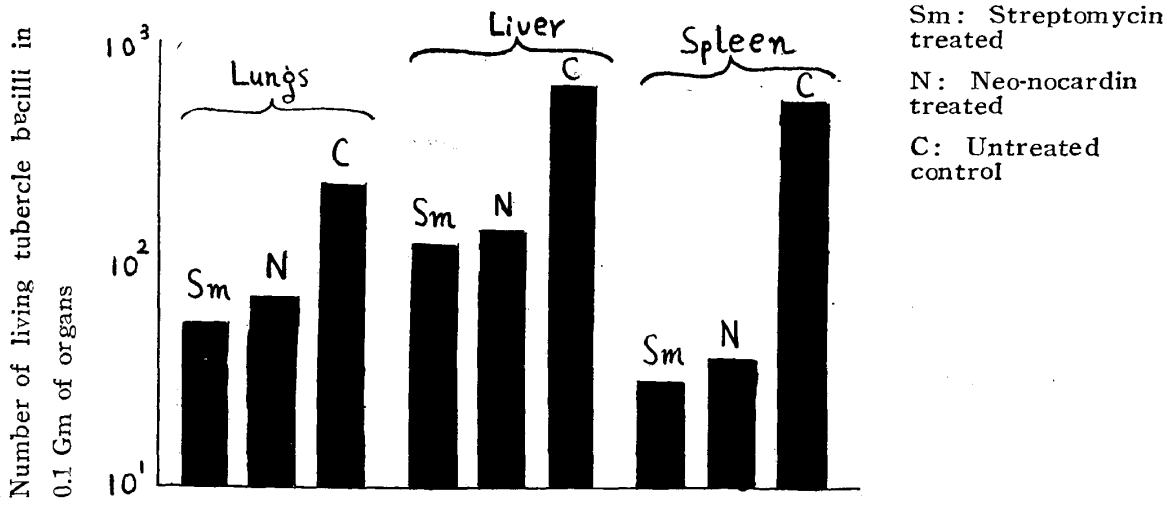
(*a*) *Administration of antibiotics was begun on the day the mice were infected and continued for a week.*

Two different experiments were done. At the first experiment each of the treated animals received daily 1.0 mg of neo-nocardin or streptomycin in two equal doses. In the control animals average number of living tubercle bacilli isolated by Ogawa's method was 3040 per 0.1 Gm of spleen and was 170 in 0.1 Gm of lungs. On the contrary, no tubercle tubercle bacilli was isolated from spleen and lungs both neo-nocardin and streptomycin treated

animals.

In the second experiment a daily dose of 1.0 mg of the antibiotics was administered in one dose. As seen in Figure 2 neo-nocardin as well as streptomycin exerted a marked antituberculous activity and there was no remarkable difference between the activity of neo-nocardin and streptomycin.

Fig. 2. Number of living tubercle bacilli.
Treatment: Began on the day of infection, continued for a week



(*b*) *Administration of antibiotics was begun a week after infection and continued for a week*

As seen in Figure 3 neo-nocardin had no remarkable effect upon the tubercle bacilli in mice. Though streptomycin seemed to have some effect upon the bacilli in mice, it was apparently less effective than in the former experiment.

(*c*) *Administration of antibiotics was begun a week after infection and continued for two weeks*

Since it was shown that the effect of antibiotics was not remarkable when these were administered a weed after infection, the treatment was paolonged for two weeks.

In this case neo-nocardin was administered in a daily dose of 2.0 mg in two equal doses. Streptomycin was administered daily 1.0 mg. in two equal doses. As shown in Figure 4 both antibiotics exerted a marked effect upon the tubercle bacilli in mice. The effecf of neo-nocardin was more remarkable than that of streptomycin. It must be mentioned, however, that the mice treated with neo-nocardin in a daily dose of 2.0 mg for two weeks lost their weight and consumed— the evidence of toxicity of crude neo-nocardin.

Fig. 3. Number of living tubercle bacilli
Treatment: Began a week atter infection, continued for a week

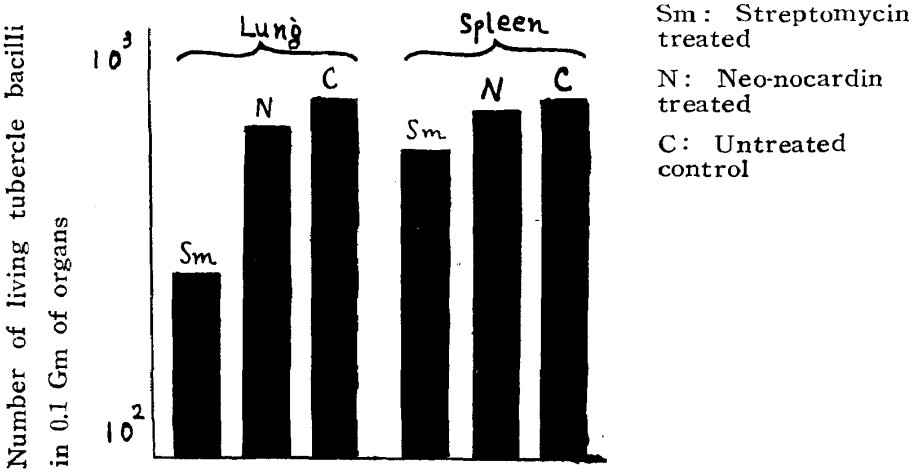
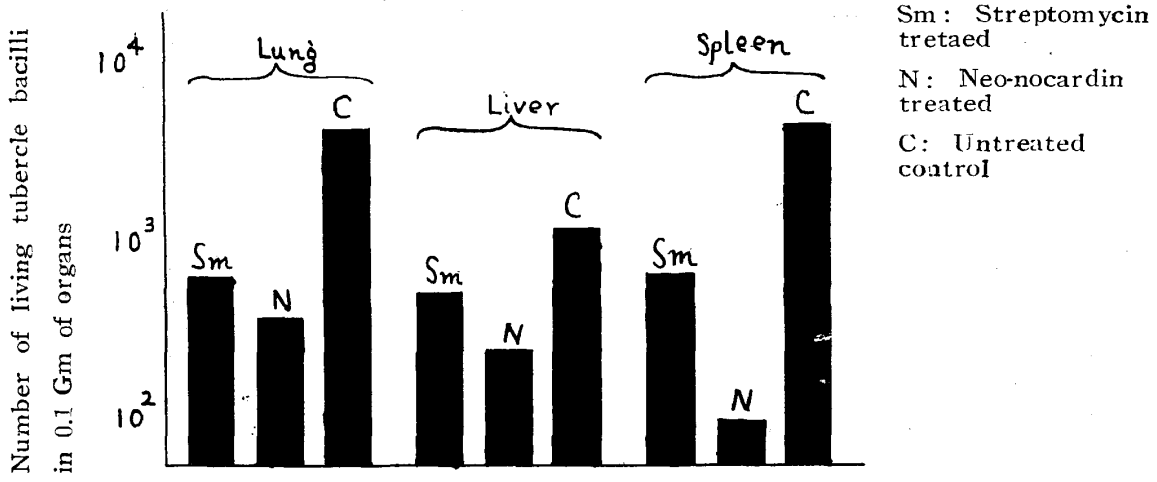


Fig. 4. Number of living tubercle bacilli
Treatment: Began a week after infection, continued for two weeks



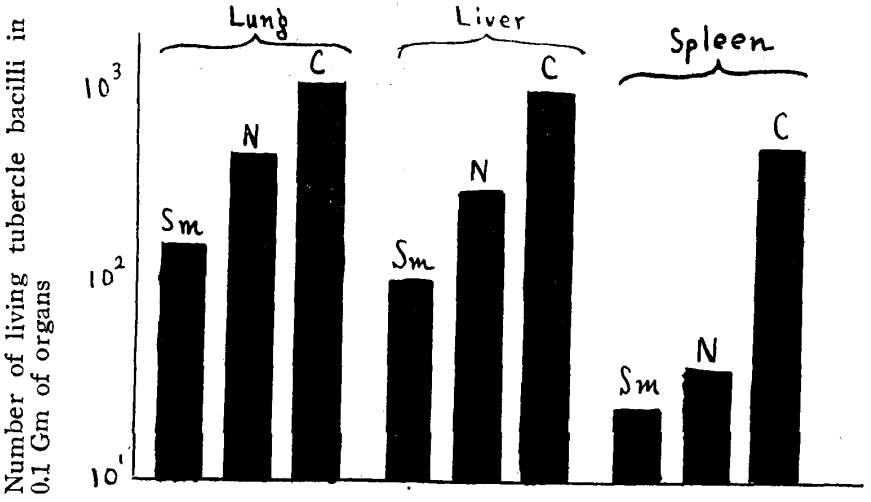
(*d*) *Administration of antibiotics was begun 6 weeks after infection and was continued for a week*

As shown in Figure 5 both antibiotics exerted some effect on the tubercle bacilli in mice.

Gross findings of mice at necropsy

None of the animals, both treated and untreated, had gross signs of tuberculosis in the organs at necropsy until 4 weeks following inoculation. Though severe involvement of lnngs was observed after 6 weeks following inoculation, tuberculous involvement of spleen and liver was less severe than that of lnngs. The weight of spleen was in general less than 100 mg

Fig. 5. Number of living tubercle bacilli
Treatment: Began 6 weeks after infection, continued for a week



Sm: Streptomycin treated
N: Neo-nocardin treated
C: Untreated control

until 1 to 2 weeks following inoculation. Increasing its weight gradually as infection progressed, some attained 350 mg of weight. Namely, in experiment (a), (b), and (c) tuberculous involvement was not found macroscopically even in untreated animals. In experiment (d) gross signs of tuberculosis were found in all animals before the treatment had begun and neo-nocardin as well as streptomycin could not effect the macroscopic findings of lungs.

Comment

The effect of neo-nocardin on the tubercle bacilli in mice was compared with that of streptomycin. It may be concluded from the above-mentioned results that there is no remarkable difference between the activities of streptomycin and neo-nocardin *in vivo*. This result is quite consistent with the one obtained *in vitro* experiment.

Summary

The effect of the crude hydrochloride extracted from the fermented broth of Nocardia kuroishi A. 422 (neo-nocardin) on the tubercle bacillus in mice was investigated.

(1) Mice were infected with tubercle bacilli intravenously and the quantitative estimation of the living bacilli in lungs, spleen and liver was done every seven days.

(2) In relation to the growth of tubercle bacilli in the organs of mice, the development of tuberculous infection may be divided into three stages.

(3) Treatment with antibiotics — neo-nocardin and streptomycin — was

done in each stages of infection. It was recognized that there is no remarkable difference in the antituberculous activity between two antibiotics tested.

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Acknowledgement

The author is indebted to Professor Dr. S. Uyeda for his kind directions throughout the work.