Effect of the Isoniazide on Guinea Pigs Infected with Isoniazide Resistant Strains of Mycobacterium Tuberculosis*

J. S. INCZE, M.D.**
Wooroloo, Australia

Introduction

The task of our present investigation was to demonstrate the action of isoniazide, if any, upon the isoniazide resistant strains of mycobacterium tuberculosis. We endeavored to find the level of resistance beyond which the drug is no longer effective and no clinical improvement is to be expected.

It is a well known fact, that a relatively high percentage of patients, treated by isoniazid after the elapse of a certain period of time, or after a certain quantity of medication develop tubercle bacilli which are resistant to this drug.1-3 We tried to demonstrate this fact by using guinea pigs as experimental animals.

Methods

We had six groups of guinea pigs, each consisting of two individuals. The guinea pigs were approximately of the same age and weight (400 gms.). We infected all six groups with 0.1 mgm. of tubercle bacilli obtained from colonies grown on Loewenstein-Jensen medium. One of the strains was sensitive to isoniazide, the others were resistant for different strengths of isoniazide concentration. We considered the first group a control, the individuals of which were infected with isoniazide resistant strain (100 mcgm./cc.). The groups 2, 3, 4, 5, and 6 were infected with approximately the same quantity of mycobacterium tuberculosis, but of different degrees of resistance.

The individuals of the second group were injected with a strain cultured from sputum of a patient who had never been treated with any antituberculous chemotherapeutic agent and whose bacilli had been proved sensitive to isoniazide, i.e. it had not been possible to subculture them in presence of isoniazide in concentration of 0.02 mcgm./cc. The third group was inoculated with micro-organisms resistant to isoniazide in concentration of 0.1 mcgm./cc. The fourth group was infected with a strain resistant to 1 mcgm./cc., the fifth group received injection with bacilli resistant to 20 mcgm./cc. and the sixth group was infected with mycobacterium tuberculosis resistant to isoniazide up to the concentration of 100 mcgm./cc.

The infected guinea pigs were injected subcutaneously in the right inner thigh on the same day, with approximately the same quantity of microorganisms and kept in separate cages from the moment of inoculation.

*From the Woorloo Sanatorium, Wooroloo, W. Australia. Medical Director, H. R. Elphick.
**Presently at Onondaga Sanatorium, Syracuse, New York.
We provided them with similar food and living conditions. Their food consisted daily of 1 oz. of dry food with the following composition: 10 per cent whole wheat, 40 per cent bran, 40 per cent crushed wheat, 5 per cent meat meal, 2½ per cent bone meal, 2½ per cent buttermilk; for every kilogram weight of dry food one teaspoonful of common salt was added. For green food and water we gave them lettuce and carrots, the quantity varied with the temperature of the air and the animals demand.

The first group served as controls and was not treated, but one of the two guinea pigs were killed on the 15th day after inoculation, to verify the progress of the infection. The other animals were examined clinically (see diagram No. 1) and we found that everyone had lymph node involvement and the animals already presented general symptoms, consisting of loss of weight and diminished appetite. The sacrificed guinea pig (first group) presented major caseation and enlargement of right inguinal lymph nodes and an enlarged para-lumbar lymph node. From the caseous lymph node we were able to demonstrate acid fast bacilli, whose size, shape and staining behavior were characteristic of mycobacterium tuberculosis.

Beginning with the 15th day, the animals of the 2–6 groups were given isoniazide, with a dose of 4.4 mgm. per kilogram of bodyweight for 10 days, after which the dosage was increased to 6 mgm. per kilogram. Fifty per cent of the drug was given orally mixed with dry food and 50 per cent by daily hypodermic injections.

---

**Diagram No. 1**

G.P. 1. Killed 15 days after infection.  
G.P. 2.  
G.P. 3.  
G.P. 4.  
G.P. 5.  
G.P. 6.  
G.P. 7.  
G.P. 8.  
G.P. 9.  
G.P. 10.  
G.P. 11.  
G.P. 12.
Results

In three to four days from the beginning of treatment we noticed that the appetite of the animals in the treated groups improved considerably, so that we had to increase the quantity of dry food to one and one-half ounces per day.

On the 15th day we examined the animals, but no significant change was found in the clinical picture, except that the treated animals ceased to lose weight.

After six weeks of treatment the animals were killed and examined. The results of examination are summarized diagrammatically in diagram No. 2.

According to diagram 2, the treatment was successful up to group 3. If we compare the pathological alterations in groups 4, 5, and 6 with the alterations of group 1 we still find that a certain difference exists between these groups. The spread of the disease in the treated groups (4, 5, 6) is not as advanced as in the untreated group 1.

The lymph nodes of the guinea pigs were examined microscopically in an endeavor to demonstrate the difference, if any, in the various groups, but no essential difference was demonstrable, between the treated and non-treated groups.
Discussion

It has been demonstrated by various writers, that patients treated by streptomycin after a lapse of time develop streptomycin resistant organisms. If the resistance reaches the level of 50 to 100 mcgm./cc. and the patients are further treated with the same antibiotic, it fails to improve the condition of the patients. On the other hand the survival of the guinea pigs infected by streptomycin resistant organisms treated by streptomycin and the untreated control groups did not show any marked difference. Other writers demonstrate that some clinical improvement could be achieved most probably owing to the fact that an increased acquired resistance occurs as a consequence of the destruction of the still sensitive strains. As we demonstrated above, the animals infected with organisms resistant to 1 mcgm. isoniazide/cc. or with micro-organisms with higher resistance than that do not respond markedly to the treatment with isoniazide or in any case not in such degree to justify the continuation of the treatment. For instance in group 2 during the treatment a generalization of the tuberculosis did not occur, the process was localized to the regional lymphatic nodes, macroscopically a certain regression was noted. On the other hand in groups 4, 5, and 6 an involvement of the spleen was evident as a sign of generalization of the tuberculous process.

Knowing that the type of tuberculosis developing in the guinea pig differs from that in the human, so the problem arises that the above statement which applies to the guinea pig may not be applicable to human tuberculosis, which develops on a substratum with a certain inborn and acquired resistance. This is a matter which has to be considered and requires further careful study and observation.

If the therapy were maintained for a longer period the difference between the groups might be more marked and further improvement could occur in the treated groups. These problems are under current investigation in this laboratory.

SUMMARY

Isoniazide administered for six weeks in the treatment of tuberculosis of the guinea pig infected with isoniazide resistant mycobacterium tuberculosis is effective to the resistance level of 1 mcgm./cc. Above this degree of resistance the progress of the disease is delayed in comparison with the non-treated control animals.

RESUMEN

La isoniazida administrada por seis semanas en el tratamiento de la tuberculosis en cuyos infectados con microbacterias tuberculosis resistentes a la isoniazida, es efectiva a una concentración de 1 mcgm./cc. Sobre este grado de resistencia la evolución de la enfermedad es retardada en comparación con la observada en los animales de control no tratados.

RESUME

Chez les cobayes inoculés avec des bacilles tuberculeux résistants à l'isoniazide, l'action du traitement par ce produit administré pendant six
semaines reste efficace lorsque le taux de résistance ne dépasse pas un gamma par cc. Au-dessus de ce degré de résistance, l'évolution de l'affection est simplement prolongée par rapport à celle des animaux témoins non traités.

REFERENCES


6 Incze, J. S.: Unpublished data.

