

PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

THE STUDY OF BACTERIOSTATIC AND CHEMOTHERAPEUTIC ACTION OF THE ANTITUBERCULOUS PREPARATION 1314 AND ITS CHLORHYDRATE

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Dr. Libermann and his co-workers [2] in France synthesized in 1956 a new antituberculous preparation, which they named 1314.

Investigations carried out by N. Rist and F. Grunbueh [1] in the Pasteur Institute in Paris showed that this preparation is effective in the treatment of experimental tuberculosis.

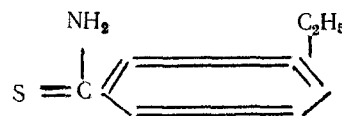
In view of the fact that the synthesis method of preparation 1314 suggested by the French investigators was found to be quite complicated, Prof. N. K. Kochetkov, N. F. Kucherovala, R. M. Khomutov and E. I. Budovskii of the Division of Organic Synthesis of the Institute of Pharmacology and Chemotherapy AMN SSSR developed in 1957 a simpler and more convenient method for synthesizing this preparation.

Realizing the low solubility of preparation 1314 in water, a method for obtaining its hydrochloride also developed.

Preparation 1314 is thiamido- α -ethylisonicotinic acid.

The empiric formula is $C_8H_{10}N_2S$.

The structural formula is

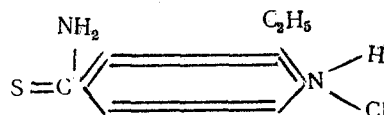


Molecular weight is 166.

The preparation is not water soluble.

The water soluble form of preparation 1314 is represented by the hydrochloride of thiamido- α -ethylisonicotinic acid. Its empirical formula is $C_8H_{10}N_2S \cdot HCl$.

The structural formula is



The molecular weight is 202.5.

In this communication we are presenting data on the study of bacteriostatic and chemotherapeutic action of preparation 1314.

EXPERIMENTAL METHOD AND RESULTS

In view of the fact that chemically the basic preparation 1314 and its hydrochloride are identical and taking advantage of the satisfactory solubility of the latter in water, we employed the hydrochloride of preparation 1314 in our in vitro experiments.

Antibacterial activity was studied in liquid bouillon medium to which the preparation was added in increasing concentrations beginning with 2 and ending with 200 μ g/ml. The microbial component for all types of bacterial cultures consisted of 250,000 microbes per 1 ml of medium.

The results of the experiments are presented in Table 1.

TABLE 1
Activity of the Hydrochloride of Preparation 1314
Regarding Various Microbes

Culture	Activity in 200 μ g/ml
Staphyl. 209	None
Staphyl. Wood	»
B. coli is	»
B. coli 35	»
B. coli resistant	»
Proteus Ox ₁₉	»
B. pyocyaneum	»
Sarcina lutea	»
B. subtilis	»
B. mycoides P-20	»
B. typhi abd. 4446	»
B. paratyphi B 42	»
B. dys. Flexneri	»
B. paratyphi A 299	»
B. dys. Sonne 714	»

As shown in Table 1, preparation 1314 in concentration of 200 μ g/ml possesses no antibacterial activity against both gram positive and gram negative microbes.

The action of hydrochloride of preparation 1314 on Mycobacterium tuberculosis was studied by us on Soton's synthetic liquid medium to which 10% of human plasma was added. The following tuberculous cultures were used in the experiment: laboratory culture type B. bovinus 8.4, and freshly isolated cultures of human types 123, 126, 122 and BCG strain.

Strain 126 was sensitive to streptomycin, phthivazide (isonicotinic acid hydrazide derivative) and PAS; strain 123 — resistant to 100 U streptomycin, 10 μ g/ml phthivazide and 50 μ g/ml PAS, strains 122 and 346 — resistant to 10 μ g/ml phthivazide and sensitive to streptomycin and PAS. In the case of each strain investigated we ran three experiments with 27 ml of microbial component to one ml of the medium. The results were recorded every seven days. Chemotherapeutic activity was noted only in those cases where controls showed a good culture growth (+++).

Data presented in Table 2 is an average of three experiments.

As shown in Table 2, preparation 1314 possesses high bacteriostatic action (in concentrations of 0.5 to 0.75 μ g/ml) on various types of Mycobacterium tuberculosis, which property brings it close to streptomycin from the chemotherapeutic standpoint.

Our attention was drawn to the fact that beginning with each third week after the initiation of the experiment a larger amount of preparation 1314 was required to maintain suppression of the growth of Mycobacterium tuberculosis. This phenomenon deserves special study.

Chemotherapeutic activity of the preparation was studied in white mice having hematogenous tuberculosis. White mice weighing 18-20 g were used. The animals were infected by the intravenous injection of 0.1 mg of B. bovinus 8 culture. Treatment was begun with preparation 1314 as well as its hydrochloride on the day following infection. Different groups of animals were given different doses and by different routes. In the controls and the treated groups there were 15 mice in each. The experimental results were evaluated by macroscopic changes in the lungs, the weight of the lungs and the number of tubercle bacilli in lung smears. All changes were recorded by the three-point system of M. V. Trius.

In Table 3 are presented average indices of macroscopic changes for each group of animals receiving preparation 1314.

As shown in Table 3, the preparation was found to be highly effective in the treatment of hematogenous tuberculosis in white mice. Indices of macroscopic changes in the lungs are low, the weight of the lungs as

TABLE 2

Action of Preparation 1314 on Mycobacterium tuberculosis

Name of strain	Record of results by days			
	7	14	21	28
Activity in $\mu\text{g/ml}$				
B. bovinus 8	—	0, 5	1	5
Human type 123 . .	—	0,75	3,5	5
Same 123	—	0, 6	3,0	7,5
» » 122	—	0, 6	3,5	7,5
» » 346	—	0, 7	3,0	5,5
BCG	—	0, 6	2,0	6,0

TABLE 3

Index of Macroscopic Changes, Weight of Lungs and the Number of Tubercle Bacilli in Lung Smears of Treated and Untreated (control) Mice

Animals	Daily dose of preparation (mg)	Method of administration	Average index of macroscopic lung changes	Average weight of lungs (mg)	Presence of tubercle bacilli in lung smears
Untreated (controls)	—	—	2,66	598	+++
1st treated group . .	1	per os	0,66	216	0
2nd » » . .	2	» »	0,73	211	0
3rd » » . .	3	» »	0,23	200	0

TABLE 4

Index of Macroscopic Changes, Weight of Lungs and Number of Tubercle Bacilli in Lung Smears of Treated and Untreated (control) Mice

Animals	Daily dose of preparation (mg)	Method of administration	Average index of macroscopic lung changes	Average weight of lungs (mg)	Presence of tubercle bacilli in lung smears
Untreated (controls)	—	—	2,66	598	+++
1st treated group . .	1	per os	0, 9	221	0
2nd » »	2	» »	0,46	220	0
3rd » »	3	» »	0,33	218	0
4th » »	2	subcutaneous	0, 5	201	0
5th » »	3	» »	0,166	188	0

compared to that in healthy animals is unchanged, tubercle bacilli are absent in lung smears. However, when the dosage was 3 mg flavado of the liver was observed at autopsy. For this reason under the conditions of the experiment doses of 1 and 2 mg per mouse must be used for therapy. Simultaneously and under the same conditions a chemotherapeutic study of the hydrochloride of preparation 1314 was conducted with administrations subcutaneously and orally. There were 15 mice in each treated and each control group. Average data on the

chemotherapeutic activity of the preparation is presented in Table 4. It must be pointed out that all treated animals survived the experiment whereas 9 of the 15 controls died between the 28th and 32nd day.

As Table 4 shows, the hydrochloride of preparation 1314 possesses high chemotherapeutic activity when administered subcutaneously and orally, which testifies to the identity of properties of preparation 1314 and its chlorhydrate.

Besides, when the preparation is administered subcutaneously in doses of 2 and 3 mg the indices of macroscopic changes in the lungs are low and equal to 0.5 and 0.166, the weight of the lungs is normal, and tubercle bacilli are absent in lung smears. An average weight gain of 2.5 g was observed in the animals during the experiment. Their survival throughout the experiment testifies to the effectiveness of the preparation.

When the preparation was administered subcutaneously in 3 mg doses some flaccidity and flavedo of the liver were found. Further investigations of chemotherapeutic properties of the preparation on animals of other species is in progress.

SUMMARY

The authors conducted an experimental study of the bacteriostatic and chemotherapeutic effect of the antituberculous preparation 1314 (thiamido- α -ethylisonicotinic acid) and its hydrochloride. The preparation appeared to be nonactive with respect to gram positive and gram negative microbes in vitro. However, it possesses a high bacteriostatic activity with respect to the *Mycobacterium tuberculosis* including certain strains resistant to other antibiotics. The chemotherapeutic effect of the preparation was checked on white mice with experimentally induced hematogenous tuberculosis. Daily administration of the preparations per os (1-2 mg per mouse) retards the development of tuberculosis. The same results were obtained with administration of the hydrochloride of the 1314 per os and subcutaneously in the dose of 2 mg per mouse.

LITERATURE CITED

- [1] Dr. N. Rist's report at the 13th All-Union Congress of Physiologists in Moscow. Manuscript.*
- [2] D. Libermann, M. Moyens, N. Rist and F. Grunbuch, Sur la préparation de nouveau thioamides pyridiniques actifs dans la tuberculose expérimentale. Comptes rendus. Des séances de l'académie des sciences, p. 242, N. 19, p. 2409, 1956.

*In Russian.