# A STUDY OF THE BACTERIOSTATIC AND CHEMOTHERAPEUTIC ACTIVITY OF THE NEW DRUG CYCLOSERINE

M. A. Berger, B. Ia. Stukalova and V. S. Mitrofanov

From the Department of Experimental Chemotherapy (Head – Doctor of Medical Sciences A. M. Shernukh) of the Institute of Pharmacology and Chemotherapy (Director – Active Member of the Acad. Med. Sci. USSR V. V. Zakusov) of the Acad. Med. Sci. USSR and the Department of Microbiology (Head – Prof. A. I. Kagramanov) of the Institute of Tuberculosis (Director – Z. A. Lebedeva) of the Acad. Med. Sci. USSR, Moscow

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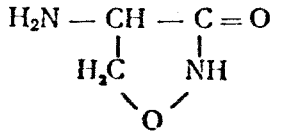
In 1956, in the department of organic synthesis of the Institute of Pharmacology and Chemotherapy of the AMN SSSR, Prof. N. K. Kochetkov and a group of his co-workers – M. Ia. Karpeiskii, R. M. Khomutov and and N. F. Kucherova [2, 3] synthesized a new chemotherapeutic drug – dl-cycloserine or 4-aminoisoxazolidone – 3.



In the present article we give the results of a study of the bacteriostatic and chemotherapeutic activity of cycloserine.

## EXPERIMENTAL METHODS AND RESULTS

We tested cycloserine by experiments both in vitro and in vivo. The antibacterial activity was studied in a liquid broth medium to which the drug was added in increasing concentrations, starting with 2 γ /ml and rising to 1000 γ/ml. The bacterial load in all cultures amounted to 250,000 microorganisms per 1 ml of medium.

The experimental results are shown in Table 1.

As shown by Table 1, cycloserine has a broad spectrum of activity. It is active against Gram-positive and Gram-negative microorganisms such as B. typhi abdominalis 4446, B. paratyphi A, B. paratyphi B, and also against various species of dysentery bacilli.

The action of cycloserine against Mycobacterium tuberculosis was studied in Soton's synthetic liquid medium with the addition of 10% human plasma. The following tuberculosis cultures were used in the experiment: a laboratory culture of B. bovinus 8, three freshly isolated cultures of the human type 123, 126, 122 and strain BCG. Strain 126 was sensitive to streptomycin, phthivazid and PAS; strain 123 was resistant to 100 units of streptomycin, 10 γ/ml of phthivazid and 50 γ /ml of PAS; strain 122 was sensitive to streptomycin and PAS and resistant to 10 γ/ml of phthivazid. For each strain which we tested we set up 3 experiments with 2 different bacterial loads in each: 27 million microorganisms per 1 ml of medium and 5.5 million microorganisms per 1 ml of medium. Readings were taken every 7 days, starting at the end of the first week, when

the control culture showed a good growth. The results, which are given in Table 2, are the mean values of the 3 experiments.

TABLE 1

Activity of Cycloserine Against Various Microorganisms

| Serial No. | Culture | Activity in γ/ml |
|---|---|---|
| 1 | Staphyl. 209 | 62.5 |
| 2 | Staphyl. Wood | 62.5 |
| 3 | B. coli 613* | 31.2 |
| 4 | B. coli 35 | 31.2 |
| 5 | B. coli (resistant) | Not active |
| 6 | Proteus $OX_{19}$ | 250 |
| 7 | B. pyocyaneum | 250 |
| 8 | Sarcina lutea | 3.1 |
| 9 | B. subtilis | 250 |
| 10 | B. mycoides P-20 | 31.2 |
| 11 | B. typhi abd. 4446 | 15.7 |
| 12 | B. paratyphi A 299 | 125 |
| 13 | B. paratyphi B 42 | 31.2 |
| 14 | B. dys. Flexneri | 31.2 |
| 15 | B. dys. Sonne 714 | 31.2 |

* Primary culture.

As may be seen from Table 2, cycloserine possesses a bacteriostatic action against Mycobacterium tuberculosis, but this activity is not high and is inferior to that of streptomycin, phthivazid and PAS. The sensitivity of different tuberculosis cultures to cycloserine is not the same. The culture bovinus 8 was found to be most sensitive to the drug, and experimental virulent strain.

It must be mentioned that cycloserine is active also against freshly isolated human strains which are resistant to streptomycin, phthivazid and PAS. Dynamic observations show the necessity of higher concentrations of cycloserine to suppress the growth of the tuberculous culture during the later stages. For this reason we set up special experiments to study the inactivation of the drug. As a result of these experiments it was clear that on standing on an incubator at 37°C for 8-10 days, cycloserine loses about 25-30% of its initial activity.

We investigated the chemotherapeutic activity of the drug in white mice with hematogenous tuberculosis and guinea pigs with tuberculosis.

For the experiment we used white mice weighing 18-20 g. The animals were infected intravenously with 0.1 mg of the culture of bovinus 8.

TABLE 2

Activity of Cycloserine Against Mycobacterium Tuberculosis

| Strains | Bacterial load in millions of microorganisms per 1 ml of medium | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 27 | | | | 5,5 | | | |
| | readings taken on days | | | | | | | |
| | 7 | 14 | 21 | 23 | 7 | 14 | 21 | 28 |
| | activity in γ/ml | | | | | | | |
| B bovinus 8 | 3.26 | 6.8 | 13.9 | 13.9 | No growth | 3.5 | 13.9 | 13.9 |
| Human type 123 | 6 6 | 17.5 | 24.0 | — | " | 8.8 | 22.2 | — |
| " 126 | 16.6 | 41.6 | 55.5 | — | 8.8 | 22.2 | — | — |
| " 122 | 9.7 | 14.9 | 27.7 | — | No growth | 7.7 | 16.6 | — |
| BCG | 4.1 | 7.7 | 14.7 | — | " | 3.2 | 11.1 | — |

Treatment began the day following infection, a dose of 0.5 mg of cycloserine per mouse being given orally. The control and treated groups each comprised 15 mice. The results of the experiments were assessed by the macroscopic changes in the lungs, the weight of the lungs and the number of tubercle bacilli in films taken from the lungs. Macroscopic changes in the lungs and the number of bacilli in the lung films were dedetermined by the three-point system proposed by M. V. Trius [4]. In Table 3 are given the mean indices for both groups of animals.

It follows from Table 3 that the drug does not show any very marked chemotherapeutic activity, although it must be noted that all the animals in the treated group were alive up to the end of the experiment. Some

Some variation is also observed in the indices of the macroscopic and bacterioscopic changes in the lungs. Of the 15 control animals, seven died between the 21st and 29th days, while all the mice of the treated group survived and were killed with the remaining living control mice on the 30th day.

The experiment to study the chemotherapeutic activity of cycloserine on guinea pigs with experimental tuberculosis was performed on 60 animals weighing 220-250 g, infected subcutaneously in the region of the right groin with 0.0001 mg of a culture of bovinus 8.

TABLE 3

Index of the Macroscopic Changes, the Weight of the Lungs and the Number of Tubercle Bacilli in Films of the Lungs in Treated and Untreated Mice

| Animals | Mean index of macroscopic changes in the lungs | Mean weight of the lungs in g | Number of tubercle bacilli in films from the lungs (index) |
|---|---|---|---|
| Treated | 1.9 | 530 | 1.6 |
| Untreated (control) | 2.3 | 568 | 2.6 |

According to the form of the treatment we divided the guinea pigs into 5 groups: groups I and III were treated with cycloserine at the rate of 5 mg orally once daily; groups II and IV were given 30 mg orally once daily; group V was the control. Treatment in groups I and II started 2 weeks after infection; in groups III and IV, 5 weeks after.

The following observations were made on the animals during the experiment: weight, inspection of reaction at the site of infection and in the regional lymphatic glands and tuberculin tests. All the animals were killed on the 117th day after infection. Macroscopic changes found at necropsy were assessed according to the system of Iu. K. Veisfeiler [1].

TABLE 4

Index of the Macroscopic Changes in the Lungs of Guinea Pigs

| Group of animals | Treatment | Mean index of macroscopic changes |
|---|---|---|
| I | 5 mg 2 weeks after infection | 16 |
| II | 30 mg 2 weeks after infection | 16,1 |
| III | 5 mg 5 weeks after infection | 16,5 |
| IV | 30 mg 5 weeks after infection | 17 |
| V (control) | | 15,3 |

From observations on the animals during the experiment no difference was observed in either the time of appearance of tuberculin sensitivity or in the change in the weight of the treated and untreated animals. As is seen from Table 4, the degree of the macroscopic changes in the animals of the different groups was also the same.

Thus under the conditions of this experiment, cycloserine is ineffective in the treatment of experimental tuberculosis in guinea pigs.

The results show that cycloserine has low toxicity towards guinea pigs, since the animals which were infected with tuberculosis tolerated well doses of the order of 30 mg per guinea pig (100-150 mg) over a prolonged period – about 100 days.

As we know, D-cycloserine obtained in the U.S.A. by a biological method is active against Mycobacterium tuberculosis in vitro [5, 9] but is not active in the treatment of experimental tuberculosis in mice and guinea pigs [7, 10]. Nevertheless D-cycloserine has been found to be a valuable drug in the treatment of various forms of tuberculosis in man [6, 8]. The reasons for this discrepancy between its chemotherapeutic activity in vitro and in vivo are not yet known. On the basis of these findings it is worth trying dl-cycloserine in hospital practice for the treatment of tuberculosis in man.

## SUMMARY

Synthetic dl-cycloserine obtained at the Institute of Pharmacology and Chemotherapy of the USSR Academy of Medical sciences was tested in experiments in vitro. It was demonstrated that this preparation has a wide antimicrobic range of action towards gram positive, as well as towards gram negative microbes, such as B. typhi abdominalis, B. paratyphi "A" et al.

Dl-cycloserine has a bacteriostatic effect toward various strains of mycobacterium tuberculosis which are sensitive or resistant to streptomycin, phthivazid and PAS.

It was revealed that this preparation does not show any significant chemotherapeutic effect in experimentally indiced hematogenic tuberculosis in mice and tuberculosis in guinea pigs. However, its toxicity in prolonged administration to guinea pigs is very low. If kept in a thermostat during 8-10 days at a temperature of 37°C the preparation is inactivited by 25 to 30%.

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