THE MECHANISM OF THE ACTION OF STEREOISOMERS OF CYCLOSERINE ON BACTERIAL CELLS

E. D. Vyshepan, K. I. Ivanova, and R. K. Ledneva

Department of Chemotherapy (Head, Professor A. M. Chernukh) Institute of Pharmacology and Chemotherapy (Director, Active Member AMN SSSR V. V. Zakusov) AMN SSSR, Moscow
(Presented by Active Member AMN SSSR V. V. Zakusov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 10, pp. 58-60, October, 1961.
Original article submitted December 22, 1960.

The problem of the mechanism of the action of stereoisomers of cycloserine, used in the treatment of tuberculosis and leprosy, has attracted a great deal of attention from microbiologists, biochemists, and clinicians. It has frequently been reported that as far as the mechanism of the action of the natural isomer D-cycloserine is concerned, on account of its structural similarity to D-alanine, it should prevent the utilization of D-alanine by the bacterium for forming the cell membrane [6, 7].

Recently, Strominger and his co-workers [8] showed that D-cycloserine acts as a competitor to inhibit the transformation of L-alanine into D-alanine, catalyzed by the phosphopyridoxal enzyme, racemase, and inhibits still more strongly the D-alanyl–D-alanine in staphylococcus aureus. Because this dipeptide is a component of the muconucleopeptide which forms part of the cell membrane, the formation of the latter is also suppressed by D-cycloserine, so that uridinenucleotidepeptide, the precursor of the muconucleopeptide, accumulates [9].

Until recently, almost nothing was known of the mechanism of action of L-cycloserine on bacterial metabolic processes. N. K. Kochepkov and his co-workers [3] suggested that D, L-cycloserine might block the phosphopyridoxal enzymes concerned in the nitrogen metabolism of the bacterial cell. To support this proposal, they quoted results which we had obtained on the suppression of transamination between the glutamic and pyruvic acids in rat liver homogenates by low concentrations of D, L-cycloserine [2]. Later, A. E. Braunshtein and his co-workers [1] showed that only two animal tissue transaminases, alanine-glutamotransaminase and the transaminase of asparagine are sensitive to L-cycloserine, and they are suppressed by low concentrations of L-cycloserine, whereas other transaminases are insensitive to both isomers. According to Buago and his co-workers [6], the alanine-glutamate-transaminase of B. coli is not suppressed even by high concentrations of L- and D-cycloserine. Thus, there is considerable disagreement between results bearing on the influence of the stereoisomers of cycloserine on this transaminase of animal tissues and bacteria.

The problem of the action of the stereoisomers of cycloserine on the activity of alanine-glutamate-transaminase is of great importance in explaining their inhibitory influence on the metabolism and growth of bacteria; we have therefore thought it necessary to investigate further this influence, and have studied it in a preparation of B-coli treated with acetone.

METHOD AND RESULTS

The results obtained are shown in the Table. Because no L-cycloserine was available, we inferred the extent of its action from the difference between the actions of D- and D, L-cycloserine.

It can be seen from the Table that there is practically no difference between the action of preparations of cycloserine on the alanine-glutamate-transaminase of B. coli and that on the corresponding enzyme obtained from animal tissue. Because D-cycloserine acts rather less strongly than does D, L-cycloserine, we think that the L-component of the racemate must be much more effective in suppressing enzymatic activity than is the D-component. We therefore have failed to confirm the findings of Buago and his co-workers. Our results also show that L-cycloserine is more effective than D-cycloserine in suppressing the assimilation of inorganic nitrogen and the synthesis of protein when B.coli is grown on a substrate containing glucose and ammonia, i. e. when the bacteria are forced to synthesize all the animo acids required for the subsequent synthesis of protein.

Possibly, the suppression of protein synthesis by both cycloserine preparations is due to the fact that they stop the formation of L-alanine in B. coli, because according to our special experiments, B. coli forms L-alanine chiefly in process of transamination between glumatic and pyruvic acids. When observing the growth of B. colion this same glucose-ammonium medium, we found that D-cycloserine caused considerable lysis of the cells, while D, L-cycloserine in the same concentration, only suppressed growth. The addition of L-cycloserine (as racemate) to D-cycloserine, as it were "protects" the cell from lysis by D-cycloserine. We never observed such a "protection' when B. coli was grown on a medium containing protein hydrolysate, but when grown on a medium lacking carbohydrate or nitrogen, D-cycloserine caused practically no cell lysis.

The Effect of Stereoisomers of Cycloserine on the Activity of Alanine-glutamate-transaminases, on the Assimilation of Inorganic Nitrogen, the Synthesis of Protein, and the Lysis of B. coli

Preparation of cycloserine	Concentration of cycloserine (in mg/ml)	Percentage suppression			Changes in the turbidity of a suspension of B. coli during growth on a glucose-ammonia medium (as a % of original value)
		Activity of alanine-glutamate-transaminase in acetone preparations of B. coli cells	Assimilation of N-NH_3 during growth on a glucose-ammonia medium	Synthesis of protein during growth on glucose-ammonia medium	
D-cycloserine	100	15	24	23	77
D, L-cycloserine	100	50	50	45	100
D, L-cycloserine + D-cycloserine	100 50		-	-	96

The following statement represents a summary of our results as affecting the influence of D- and D, L-cycloserine on the activity of alanine-glutamate-transaminase, the assimilation of inorganic nitrogen, and the synthesis of protein and other growth of B. coli. L-cycloserine usually suppresses the formation of L-alanine through transamination. Consequently, if the micro-organism cannot form L-alanine in some other way, the suppression of the activity of the alanine-glutamate-transaminase leads in turn to the suppression of protein synthesis and to the formation of uridinenucleotide-peptide, containing L-alanine. Supporting evidence is provided by an observation of Khan and his co-workers, who showed that the growth of B. subtilis, which is able to form alanine by direct amination of pyruvic acid [4], is almost insensitive to L-cycloserine. Consequently, in B. coli and B. subtilis, the sensitivity to L-cycloserine is determined by the action of transaminase in the formation of alanine. The exceptional sensitivity of the tuberculosis bacillus to L-cycloserine, may, from our view, also be due to the suppression by it of such transaminases as alanine-glutamate-transaminase, and the transaminase of asparagine which are so sensitive to it. We are at present investigating the part played by these enzymes in the vital processes of the bacterial cell.

D-cycloserine has only a small suppressive effect on the formation of L-alanine through transamination; it weakly suppresses protein synthesis, and, evidently, the formation of uridinenucleotide-peptide. Its bacteriostatic and lytic action is probably due to the suppression of the formation of D-alanyl D-alanine, as discovered by Strominger and his co-workers, substances which take part in the construction of the membrane of the bacterial cell.

The formation of a cell membrane by the action of D-cycloserine appears to be more completely suppressed than is the synthesis of protein, and this fact explains the lysis of cells under the influence of D-cycloserine [5]. We can now understand why L-cycloserine causes no lysis, although it inhibits protein synthesis in sensitive bacteria to the same extent as it does synthesis of the cell membrane.

SUMMARY

A study was made of the effect produced by D- and D, L-cycloserine on the activity of alanine-glutamate-transaminase of B. coli preparations treated with acetone, and on nitrogen assimilation, protein synthesis and B coli lysis cultivated on a glucose-ammonium medium. It was found that in its inhibitory effect on the activity of a transaminase and on protein synthesis, D, L-cycloserine is stronger than D-cycloserine. In a synthetic medium, L-cycloserine "protects" the bacterial cells from lysis by D-cycloserine. It is suggested that D-cycloserine inhibits the formation of the cell membrane more strongly than protein synthesis, whereas L-cycloserine inhibits both processes equally; also that the action of L-cycloserine on the sensitive bacteria is probably caused by the suppression of the effect of some transaminase.

LITERATURE CITED

1. R. M. Azarkh, A. E. Braunshtein, T. S. Paskhina., Syui Tin-sen, Biokhimiya, 25, No. 5 (1960), p. 954.
2. E. D. Vyshepan, K. I. Ivanova, and A. M. Chegnukh, Byull.Éksper. Biol. i Med., 48, No. 8 (1959), p. 52
3. N. K. Kochetkov, R. M. Khomutov, M. Ya. Karpeiskii, et al., Dokl. AN SSSR, 126, No. 5 (1959), p. 1132.
4. Khun'Mun-min, Shen'San-chun, and A. E. Braushtein, Biokhimiya, 24, No. 5 (1959), p. 929.
5. J. Park, in the book: The Strategy of Chemotherapy [in Russian]. (Moscow, 1960), p. 63.
6. A. Buago, A. DiMarco, M. Chione, et al., G. Microbiol. 6 (1958), p. 131.
7. J. Ciak, and F. Hahn, Antibiot. and Chemother., 9 (1959), p. 47.
8. J. Strominger, E. Ito, R. Threnn, J. Am. chem. Soc., 82 (1960), p.998.
9. J. Strominger, R. Threnn, and Sh. Scott, 81 (1959), p. 3803.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
