BIOLOGICAL PROPERTIES OF GLUTAMIN(ASPARAGIN)ASE FROM *Pseudomonas boreopolis* 526

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Data in the literature on enzyme therapy indicate that enzymes are sufficiently effective antitumor agents [1, 5, 11, 12]. At the same time, they have certain disadvantages, associated both with the narrow spectrum of their antitumor action and with their rapid clearance from the blood serum, their immunogenicity, and other undesirable properties which limit their wider use in oncologic practice.

Steps to improve the chemotherapeutic properties of enzymes, including deaminases, are currently being taken in two main directions. First, research into chemical modification of enzymes, leading to increased stability of enzyme preparations in the body as a whole, is in progress. Second, a search is being made for new enzymes possessing a broader spectrum of antitumor action, greater stability, and weaker antigenicity.

In recent years the attention of research workers has been drawn to a new enzyme preparation glutamin(asparagin)ase which, compared with commercial preparations of L-asparaginases currently used in oncology, has a broader spectrum of antitumor action [11, 15].

In the present investigation the biological, and in particular, the antileukemic properties of glutamin(asparagin)ase from *Pseudomonas boreopolis* 526, isolated at the Department of Biochemistry of the Patrice Lumumba Peoples' Friendship University, were studied in experiments on mice. Previously, in conjunction with the staff of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, it was shown that the preparation in experiments *in vitro* has a marked cytotoxic action on a number of lymphatic leukemias in tissue culture [3].

EXPERIMENTAL METHOD

Three enzyme preparations were tested in the experiments: L-asparaginase from *E. coli* (from Bayer, West Germany), L-asparaginase from *E. coli* of USSR origin (these are commercial preparations, the standard pack contained 10,000 U of the lyophically dried preparation), and also the glutamin(asparagin)ase obtained at the Department of Biochemistry. The preparation was freeze-dried, and according to the results of disk electrophoresis in polyacrylamide gel and sedimentation analysis it was homogeneous and had specific activity of 55 U relative to asparagin and 79 U relative to glutamin.

The half-life of the enzyme in blood plasma was determined in experiments on mice. The enzyme was injected intraperitoneally in a concentration of 1000 U/kg and in a volume of 100 µl. The presence of deaminase activity in the animals' blood plasma was determined by micronesslerization [10].

Chinchilla rabbits weighing 2.5 kg were used for immunization. The enzyme preparations were dissolved in 0.85% NaCl solution and injected intraperitoneally and into the marginal vein of the ear. Reimmunization was given every 8 days for 45 days. The serum antibody titer was determined by Ouchterlony's method of double immunodiffusion in agarose, in Gusev's modification [2]. Cross-antigenicity of the enzymes was determined by the same method.

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TABLE 1. Dependence of Antitumor Action of Deaminases on Their Half-Elimination Time from the Blood Stream

Enzyme	Source from which obtained	Half-elimination time from blood stream, h	Antitumor activity	Literature citations
GA	Ps. geniculata	0,5	—	[8]
GA	Ac. gluraminasificans	0,9	—	[9, 13]
GA	Scinetobacter	2,0 — 3,0	+	[7]
GA	Ps. species 7A	13,0	++	[10, 11]
GA	Ps. fluorescens	6,0	++	[9]
GA	Ps. acidovorans	1,5 — 3,0	++	[6]
A	E. carotovora	4,0	++	[9]
A	S. marcescens	2,4 — 4,0	++	[4]
GA	Ps. vulgaris	1,5	+	[9]

Legend. GA) Glutamin(asparagin)ase, A) L-asparaginase.

TABLE 2. Antitumor Action of Deaminases on P-388 Lymphocytic Leukemia

Preparation	Dose, U/kg	Lengthening of life, % of control	
		experiment 1	experiment 2
Glutamin(asparagin)ase	1000	—30	—34
	500	50	73
	250	30	30
	150	30	30
L-asparaginase (USSR)	1000	—0	0
	500	—16	0
	250	0	0
	150	—10	—5
L-asparaginase (Bayer)	1000	—13	0
	500	—7	0
	250	0	0
	150	—19	0

The antitumor action of the deaminases was studied in BDF_1 mice with experimental P-388 lymphatic leukemia. The deaminase preparations were dissolved in distilled water immediately before use and injected intraperitoneally into the animals 24 h after inoculation of the leukemia in doses of 150 to 1000 U/kg for 5 days. The criterion of effectiveness of the compounds was an increase in the length of survival of the animals treated with the preparation, compared with this parameter in untreated mice. The action of specific antibodies on enzyme activity was studied by incubating the enzyme with specific antisera at 37°C for 1 h, after which aliquots were taken from the reaction samples and residual specific activity of the enzyme was measured.

EXPERIMENTAL RESULTS

One of the important properties of the enzyme preparations, responsible for their antitumor action, is the length of time they remain in the body in an active state. Date on antitumor activity and the half-life of the enzyme are summarized in Table 1; they show that some preparations of deaminases had no antitumor activity whatever. As a rule these enzymes had a short half-life in the plasma.

The results of the experiments to study clearance of glutamin(asparagin)ase from *Ps. boreopolis* 526 are shown in Fig. 1. The half-life of the enzyme in mouse blood serum was 8.5 h, which is twice as long as that of L-asparaginase from Bayer, and also higher than that of preparations of glutamin(asparagin)ases of different origin. However, it must be pointed out that our preparation was inferior with respect to this parameter only compared with the enzyme from *Ps. species* 7A (Table 1). The highest concentration of the enzymes compared in the animals' blood plasma was observed 2 h after injection. However, when L-asparaginase was used, a gradual fall of its concentration was noted almost immediately after injection, and after 9 h

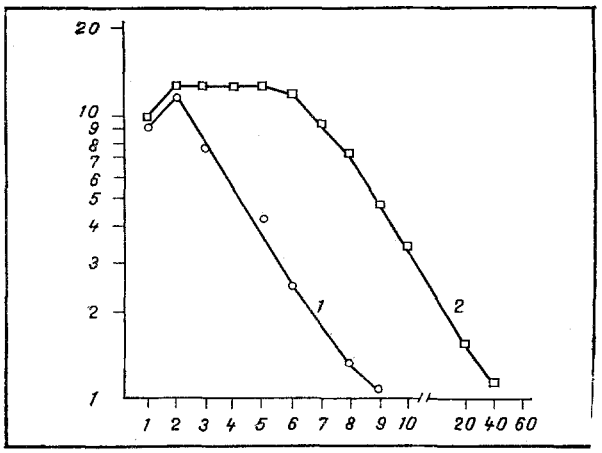

Fig. 1. Determination of half-elimination time of glutamin(asparagin)ase from mouse blood plasma. Abscissa, time (in h); ordinate, plasma enzyme activity (in U/ml). 1) L-asparaginase (Bayer); 2) glutamin(asparagin)ase.

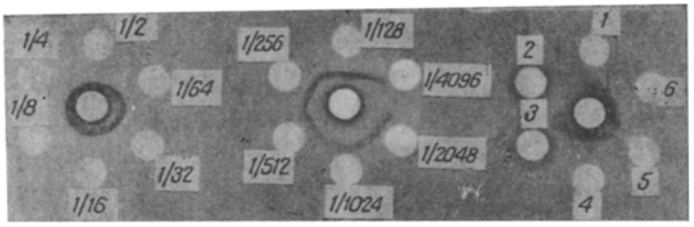

Fig. 2. Immunodiffusion reaction in agarose: a, b) Determination of titer of specific antibodies against L-asparaginase (Bayer, West Germany); c) crossed-antigenicity. 1, 5) Wells with glutamin(asparagin)ase in different concentrations; 4) well with L-asparaginase from Bayer; 5) well with L-asparaginase from USSR.

Note. Reactions a, b, and c not identified in Russian original.

activity of the enzyme in the plasma was hardly detectable. Activity of glutamin(asparagin)ase from *Ps. boreopolis* 526 in the plasma showed hardly any decrease at all for 3 h. After this, however, its concentration gradually began to fall, and 40 h after its injection, low but still measurable enzyme activity was detected in the plasma.

We know that during repeated courses of treatment with deaminases their antitumor activity declined sharply, but the sensitivity of the recipient to the preparation increases, due to elevation of the antibody titer.

Special investigations showed that glutamin(asparagin)ase from *Ps. boreopolis* 526 has relatively low immunogenicity. The precipitation test in agarose showed that the blood antibody titer of rabbits immunized with preparations of glutamin(asparagin)ase for 6 weeks was 1:64 (for crasnitin it was 1:256). It will be clear from Fig. 2 that specific antibodies obtained against L-asparaginase (Bayer) do not affect activity of glutamin(asparagin)ase but sharply reduce the activity of preparations of both L-asparaginases. Antibodies to glutamin-(asparagin)ase depress activity of the latter. The degree of the inhibitory action of antibodies on enzyme activity depends on the blood antibody titer.

One of the main conditions for successful use of deaminases as therapeutic substances is their relatively long administration and the possibility of giving repeated courses of treatment. As was pounted out above, during long-term administration of the same enzyme, its activity decreases or disappears, in connection with the development of immunologic reactions. It is therefore important to have immunologically different enzyme preparations available.

In the present investigation cross-antigenicity of commercial preparations of deaminases and of glutamin(asparagin)ase from *Ps. boreopolis* 526 was tested by immunodiffusion in agarose. The commercial preparation of L-asparaginase from Bayer was found not to exhibit cross-antigenicity with the glutamin(asparagin)ase, whereas it formed precipitation bands with L-asparaginase from *E. coli* of Soviet origin (Fig. 3).

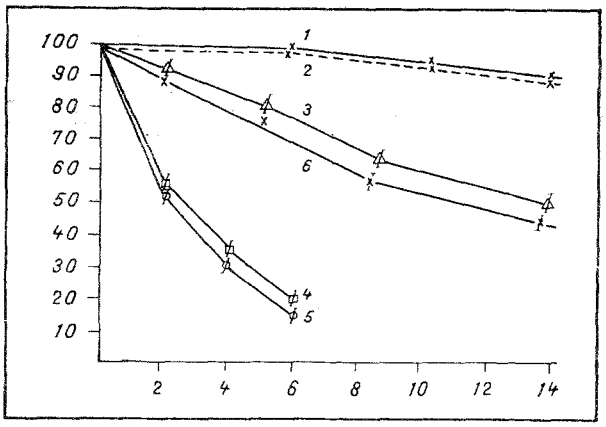

Fig. 3. Action of specific antibodies on catalytic activity of L-asparaginases and glutamin(asparagin)ase. Abscissa, time (in h); ordinate, relative activity (in %). 1) Glutamin(asparagin)ase in mouse blood plasma (control); 2) effect of specific antibodies (titer 1/256) against L-asparaginase (Bayer) on glutamin(asparagin)ase activity; 3) effect of specific antibodies (titer 1/64) against glutamin(asparagin)ase on glutamin(asparagin)ase activity; 4) effect of specific antibodies (titer 1/256) against L-asparaginase (Bayer) on activity of L-asparaginase (USSR); 5) effect of specific antibodies against L-asparaginase (Bayer), titer 1/256, on activity of L-asparaginase (Bayer); 6) effect of specific antibodies against L-asparaginase (Bayer), titer 1/64, on activity of L-asparaginase (Bayer).

In the final series of experiments the antitumor action of deaminases was studied on experimental lymphatic leukemia P-388 in experiments *in vivo* (Table 2). The glutamin(asparagin)ase which was tested was found to have marked antitumor properties. The optimal dose for leukemia P-388 was 500 U/kg. Since the other deaminases tested, and in particular, L-asparaginase (Table 2) have no analogous action on this type of leukemia, it must evidently be postulated that the effect of the deaminases was most probably linked with their glutaminase, and not with their asparaginase activity, by analogy with their action on lymphatic leukemia L-1210, whose sensitivity is similar [11].

The results show that glutamin(asparagin)ase from *Ps. boreopolis* 526 differs immunologically from commercial preparations of L-asparaginases, it has a longer half-life in the plasma, and has an antitumor effect on lymphatic leukemia P-388, on which preparations of L-asparaginases have no action.

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