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SENSITIVITY OF AML LEUCOCYTES IN CULTURE TO L-METHIONINASE

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L-methioninase (L-methionine-α-deamino-γ-mercaptomethane-lyase)(EC4.4.1.11) isolated from Clostridium sporogenes (1) has been found to inhibit the growth of P815 cell cultures and Walker carcinosarcoma of the rat, which indicates the therapeutic usefulness of this enzyme (2). In our studies with L-methioninase isolated from Pseudomonas putida AC-75 we have found (3) that the enzyme at the concentration of 0.001-0.004 U/ml of the medium produced a 50% inhibition of growth of L 5178Y mouse lymphoblasts in culture.

The purpose of this study was to determine the sensitivity to the enzyme of leucocytes isolated from blood samples of patients with acute myeloblastic leukemia.

MATERIALS AND METHODS

Patients with leukemia

In this study 12 patients with acute leukemia were analysed. All cases were classified as acute myeloblastic leukemia (AML) on the basis of the results of cytochemical reactions according to the accepted criteria.

L-methioninase (L-methionine-α-deamino-γ-mercaptomethane-lyase)

The enzyme was extracted from Pseudomonas putida AC-75 by sonic disruption. In the purification procedure the following steps were used: removal of nucleic acids with protamine sulphate, fractionation with ammonium sulphate, chromatography on DEAE Sephadex A-50 and thermic denaturation of impurities. The specific activity of the obtained preparation was 0.107-0.163 U/mg protein. All purification steps were performed at room temperature in the presence of pyridoxal phosphate (Fluka, Buchs, Switzerland) and dithiothreitol (Koch-Light, Colnbrook, England) . Samples of the semipurified enzyme were sterilized by filtration through Millipore filters. One unit of enzyme is defined as the amount of enzyme that catalyzes the formation of 1 μmol of methanethiol per minute at 30° C according to the method of Ito et al (4). The enzyme was dissolved in a phosphate buffer (pH 7.4) with pyridoxal phosphate. Solutions containing 1U of L-methioninase per ml of this buffer were employed. The experiments were performed with freshly prepared samples.

Cell cultures

The cell suspensions were prepared from the buffy coat of the peripheral blood. Leucocytes were washed three times and suspended in RPMI 1640 medium (Gibco, Paisley, Scotland) supplemented with 15 % (v/v) calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells (1.2×10^6) were incubated in 2 ml of this medium or in the same medium but containing L-methioninase at concentrations of 0.001; 0.005; 0.01; 0.05 U/ml of medium.

Evaluation of the response

The sensitivity of leukemic cells to L-methioninase was estimated on the basis of differences in the capability to incorporate tritiated valine and differences in the capability to survive. L [^{3}H] valine (1 μCi/ml - 2 Ci/mmole, Amersham, Searle, USA) was added to each culture just before the start of incubation. The cultures were incubated at 37° for 34 h. At certain time intervals the incubation was interrupted in a given number of tubes, and incorporation of [^{3}H] -radioactivity into acid-insoluble material was determined by liquid scintillation spectrometry (5). Trypan blue exclusion was employed to determine cell viability.

RESULTS AND COMMENTS

Fig. 1 and 2 illustrate graphically the diverse response of L-methioninase treated AML leucocytes. In the case of sensitive leucocytes (Fig 1) there was a relationship between response and concentration of the enzyme in the culture medium. From a comparison of the curves in Fig. 2 it is evident that leucocytes derived from the other samples were resistant since they did not respond to the action of L-methioninase, even at the concentration of 0.05 U/ml of the medium.

Table 2 shows the results of determinations of sensitivity of leucocytes from 12 patients after treatment of cells with L-methioninase at the concentration of 0.005 U/ml for 34 h. In the same table, hematologic and therapeutic data are presented. In 5 cases of untreated and in 2 cases of treated samples AML incorporation of L [^{3}H] valine was in the range of 60 - 20 % of the control value. Cells from 5 other drug-treated patients incorporated an equal or similar amount of valine, regardless of the presence or absence of L-methioninase. In general, differences in the ability of leucocytes to incorporate tritiated valine corresponded to the differences in their capability to survive.

In summary, the experiments performed on cultures of leucocytes isolated from 12 patients with AML indicate that samples derived from 7 patients showed a high degree of sensitivity to L-methioninase from Pseudomonas putida AC-75. On the basis of these preliminary studies it is not possible to explain the unresponsiveness of leucocytes derived from 5 cases which were classified as the same kind of leukemia.

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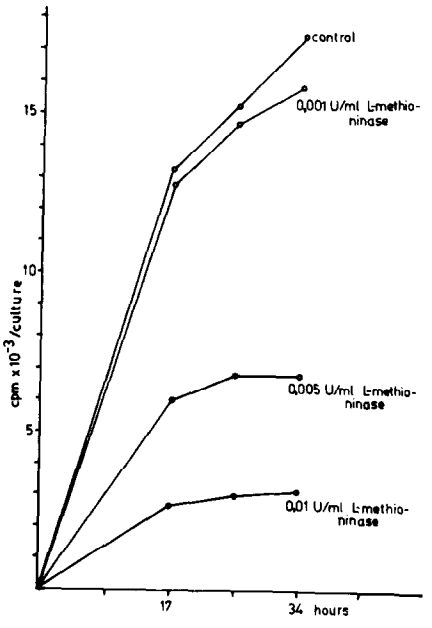

Fig. 1. Effects of L-methioninase treatment on L[^{3}H] valine incorporation in cultured leucocytes from patient No 5. A similar response was obtained in cases No 1,2,3,4, 8 and 10. The experiments were performed as indicated in Materials and Methods. Results are mean values from determinations in three or four cultures.

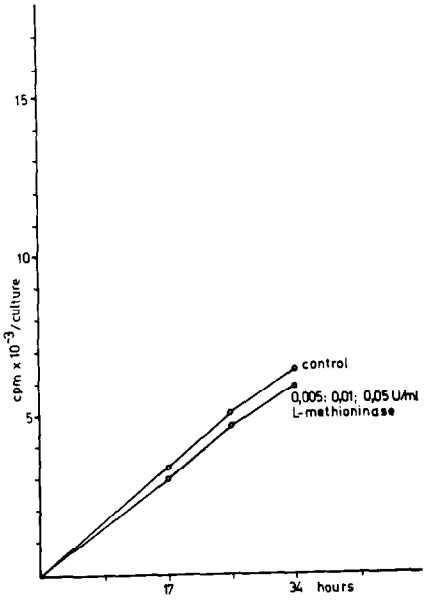

Fig. 2. Effects of L-methioninase treatment on L [^{3}H] valine incorporation in cultured leucocytes from patient No 9. A similar response was obtained in cases No 6, 7, 11 and 12. The experiments were performed as indicated in Materials and Methods. Results are mean values from determinations in three or four cultures.

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Table 1

Sensitivity of peripheral AML leucocytes to L-methioninase

Case No	Sex	Age	White blood cells G/l	Percent myelo-blasts	Therapy [x]	% inhibition [+] L-methioninase 0.005 U/ml	% survival [++] L-methioninase 0.005 U/ml
1.	F	22	5.1	47	No treat-	50	N.T.
2.	F	26	4.6	41	ment	70	N.T.
3.	M	26	4.9	41	"	68	35
4.	F	20	5.1	33	"	40	65
5.	F	32	16.6	59	"	60	30
6.	F	34	1.5	4	VCR,AraC, MTX,6-MP, Pred.	0	100
7.	M	27	2.1	0	CP,VCR, AraC,Pred.	13	80
8.	F	28	0.5	0	"	65	50
9.	M	56	5.8	29	VCR,ThG, Adr,MTX, 6-MP	0	100
10.	M	55	2.6	6	CP,VCR, AraC,Pred.	80	42
11.	F	55	5.2	34	CP,VCR, AraC	0	100
12.	F	28	2.5	8	AraC,Adr, MTX,6-MP, Pred.	0	100

Abbreviations used: VCR - vincristine; AraC - cytosine arabinoside; MTX - amethopterine; 6-MP - 6-mercaptopurine; CP - cyclophosphamide; ThG - thioguanine; Adr - adriamycin; Pred - prednisone.

N.T.- not tested

x - cells from treated patients were taken during treatment

+ - percent inhibition of protein synthesis after 34-hr incubation as compared to 100 % of control

++ - percent of cell survival after 34-hr incubation as compared to control taken as 100 % survival