EFFECTS OF ENZYME INHIBITORS IN INHIBITING THE GROWTH AND INDUCING THE DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS, HL-60

Sir:

It is well known that bestatin binds to cells, most strongly to macrophages[1] and enhances immune responses[2~4]. It has also been reported that the addition of bestatin treatment to chemotherapy prolonged the survival periods of patients of nonlymphocytic leukemia[5], melanoma[6] *etc.* Recently OKUYAMA[7] reported that bestatin caused a differentiation of cancer cells. Therefore, it became necessary to clarify whether the action causing differentiation is involved in the therapeutic effect of bestatin. Besides bestatin, arphamenines A and B[8], inhibiting aminopeptidase B, amastatin[9], inhibiting aminopeptidase A, forphenicine[10], inhibiting alkaline phosphatase and forphenicinol[11] (a derivative of forphenicine) have been found in this institute to be immuno-modifiers. Therefore, we examined their action in inhibiting the growth and inducing the differentiation of human promyelocytic leukemia cells, HL-60[12].

HL-60 cells were cultured in RPMI1640 medium supplemented with 15% of fetal calf serum (Grand Island Biochemical Co., Grand Island, N.Y.) and gentamicin (80 μg/ml) at 37°C in an atmosphere of 5% CO_2 in air in humidified incubators. The HL-60 cells were obtained from Dr. HOZUMI (Saitama Cancer Center Research Institute). As the marker of cell differentiation to granulocytes, intracellular blue-black formazan deposits (BFD) produced by reduction of nitro blue tetrazolium (NBT)[13] was employed. After culturing with the test materials described above for 5 days, the cells were washed with phosphate-buffered saline and incubated in RPMI1640 containing 0.1% NBT and 100 ng/ml phorbol 12-myristate 13-acetate, for 20 minutes at 37°C. After staining with May-Grüenwald-Giemsa, the percentage of cells containing BFD was counted under light microscopy; at least 200 cells were examined for each experiment. The concentrations of the test materials except forphenicine were 12.5, 25, 50 and 100 μg/ml. The concentrations of forphenicine were 1.25, 2.5, 5 and 10 μg/ml. The growth was examined by Coulter Counter (Coulter Electronics Ltd., England). The results are shown in Tables 1 and 2.

Table 1. Effect on growth and differentiation in HL-60 cells by proteinase inhibitors.

Drugs	Concentration (μg/ml)	% of control cells	% of BFD containing cells
Arphamenine A	100	19.1	59.0
	50	51.3	18.7
	25	76.1	9.1
	12.5	87.1	4.9
Arphamenine B	100	43.4	33.6
	50	58.2	15.8
	25	71.8	10.2
	12.5	84.3	2.3
Bestatin	100	38.3	24.6
	50	47.3	13.7
	25	50.3	9.5
	12.5	58.6	7.8
Amastatin	100	91.3	1.3
	50	97.7	1.8
	25	104.0	2.8
	12.5	107.0	1.3
Control	0	100.0	1.7

HL-60 cells were cultured at various concentration of drugs in medium for 5 days.

Table 2. Effect on growth and differentiation in HL-60 cells by enzyme inhibitors.

Drugs	Concentration (μg/ml)	% of control cells	% of BFD containing cells
Forphenicine	10	23.3	49.2
	5	73.7	3.0
	2.5	98.5	4.1
	1.25	98.6	3.3
Forphenicinol	100	102.0	1.4
	50	102.0	2.4
	25	107.0	1.1
	12.5	108.9	0.6
Control	0	100.0	1.0

Arphamenine A at 100 μg/ml showed a strong growth inhibition, and at this concentration 59.0% cells contained BFD. Arphamenine B showed weaker activity both in growth inhibition and in differentiation induction than does arphamenine A. Bestatin had further weaker activities in both actions. Amastatin at 100 μg/ml had almost no activity. Forphenicinol at 100 μg/ml had also almost no activity. Forphenicine was the strongest in both activities, because its 10 μg/ml produced 77% growth in-

hibition and 49.2% differentiation induction. As the whole, it can be said that the growth inhibition always occurred in the condition where the differentiation was observed.

The time courses of the cell growth and percentage of cells containing BFD during 7 days after the addition of arphamenine A at 100 μg/ml was examined. In this case also, the increase of the percentage of differentiated cells was accompanied by the inhibition of cell growth.

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