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ACTION OF HYPOTHALAMIC HORMONE ON NUDE MICE WITH TRANSPLANTABLE STRAINS OF HUMAN TUMORS

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Melanostatin (the tripeptide propyl—leucyl—glycinamide, H—Pro—Leu—Gly—NH_2), a hypothalamic hormone which inhibits the melanocyte-stimulating hormone of the pituitary gland, has been synthesized at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. The targeted synthesis of hypothalamic hormones became possible once their peptide nature had been established [6].

Considering the role of melanostatin in melanin formation, it was natural to suggest its action on melanomas. This paper discusses the results of a chemotherapeutic and cytological study of melanostatin on models of melanoma and other human tumors.

EXPERIMENTAL METHOD

Experiments were carried out on 6- to 8-week nude mice (based on line BALB/c) with subcutaneously inoculated (with a suspension of tumor cells in Hanks' solution) strains of human tumors. Melanostatin was dissolved in distilled water and injected into mice with melanoma (Mel-1) on the 10th day after transplantation (when the average volume of the tumors was 0.3 cm^3) in a dose of 70 mg/kg intraperitoneally, twice with an interval of 72 h between injections. Inhibition of tumor growth was calculated as the difference in their volume in the control and treated groups. The morphological composition of the peripheral blood was determined 3 days after the end of treatment: the functional state of the blood lymphocytes and tumor cells (in squash preparations of the tumors) was assessed cytochemically as succinate dehydrogenase (SDH) and α-glycerophosphate dehydrogenase (α-GPDH) activity, as described previously [1]. Fluorescein sodium (FlNa) was injected into retro-orbital sinus on the 10th day, 1 h before sacrifice of the mice with Mel-1; the method described in [2] was used for the investigation. Accumulation of FlNa was determined in squash preparations of the tumors and organs (liver, spleen, lungs, kidney) of the control and treated mice.

Accumulation of FlNa also was estimated after contact exposure of strains of melanoma (Mel-5), lung cancer (LC-1), and carcinoma of the liver (CLi), obtained from material removed at operation, to melanostatin in vitro. The strains were studied in the period of maximal tumor growth on the 14th-20th days after transplantation. Fragments of tumor (control and experiment) were incubated in Hanks' solution with FlNa for 5 min at 37°C, after which they were made.

The degree of fluorescence after injection of FlNa was determined under the "Lyumam I-3" microscope at $\lambda = 516 \pm 16$ nm, with FMÉL1-A photometric attachment and the Sh-4300 combined digital computer. The intensity of fluorescence of each squash preparation of tumors and organs was determined in ten fields of vision (U = 2v).

The results were subjected to statistical analysis by nonparametric tests [3].

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TABLE 1. Action of Melanostatin on Strain of Human Melanoma

Group	Percent inhibition of tumor growth		Leukocytes, $\times 10^3$	Lymphocytes, $\times 10^3$	Neutrophils, $\times 10^3$	Enzyme activity per cell, units			
						lymphocytes		tumor	
	after 5 days	after 10 days				SDH	α-GPDH	SDH	α-GPDH
Control			10.0	6.6	3.1	10.2	9.9	17	21
Experimental	58*	56*	6.4*	2.8*	3.4	10.7	10.0	14	19

Legend. Here and in Tables 2 and 3: *denote significance of difference relative to control ($p < 0.05$).

EXPERIMENTAL RESULTS

Melanostatin was synthesized by classical methods of peptide chemistry, based on gradual lengthening of the chain, starting at the C-end. The results of investigations of melanostatin on mice with Mel-1 are given in Table 1. Strain Mel-1 was obtained from a cell line, and in the control the average life span of the mice was 60 days. During the ten days after injection of melanostatin moderate inhibition of growth of the melanoma was observed, and its volume at this time in the control reached 8.4 cm^3, falling to 3.6 cm^3 after treatment. Moderate lymphocytopenia was observed: the lymphocyte count was 42% of the control value, but the number of polymorphonuclear leukocytes was unchanged.

The level of SDH and α-GPDH activity per peripheral blood lymphocyte after treatment was virtually the same as in the control, evidence of the absence of any direct inhibitory action on enzymes of energy metabolism in the lymphocytes. Meanwhile, in the melanoma cells a tendency was observed for activity of these enzymes to fall, and this could indicate a certain selective inhibition of SDH, reflecting the state of oxidative processes in the Krebs' cycle, and of α-GPDH, responsible for the linking of glycolysis with respiration, in the melanoma cells compared with peripheral blood lymphocytes.

Besides the traditional evaluation of the therapeutic action of preparations based on percentage inhibition of tumor growth, the action of melanostatin also was estimated on the basis of the intensity of luminescence of the cells after injection of FlNa. The use of luminescence microscopy to study the state of cell function on the basis of accumulation of various dyes has been known since the 1950s. In particular, selective accumulation of fluorescein by tumors has been used clinically for diagnosis [4].

Values obtained for fluorescence of FlNa in melanoma cells and mouse organ cells are given in Table 2. The intensity of fluorescence of tumor cells was significantly greater than that of organ cells ($p < 0.05$), confirming the view that FlNa accumulates selectively in tumors. The degree of fluorescence of melanoma cells after treatment was increased by 50%, and it correlates with the degree of inhibition of its growth. Reduction of FlNa accumulation after treatment in the kidneys and lungs (by 75%) may probably indicate affinity of melanostatin for these organs.

The significant difference in fluorescence between the control and experimental specimens on contact exposure of the tumors to melanostatin and FlNa (Table 3) is in agreement with the view that this parameter can be used to assess the action of therapeutic preparations.

The intensity of fluorescence of the melanoma after treatment with melanostatin was reduced by 40% compared with the control. Accumulation of FlNa under the influence of melanostatin also was reduced by the same degree in lung cancer and by a lesser degree in the case of carcinoma of the liver (by 30%).

Thus the hypothesis on the action of melanostatin on melanoma was confirmed under experimental conditions, as shown by the consistent trend in the percentage of inhibition of tumor growth, reduction of accumulation of FlNa by the treated tumors, and the course of inhibition of energy metabolism of the tumor cells. The antitumor effect was accompanied by moderate lymphocytopenia, by absence of any direct inhibitory action on enzymes of energy metabolism in the lymphocytes, and by a marked fall in the degree of fluorescence in the kidneys and lungs. The difference observed in uptake of FlNa by the melanoma and lung cancer can be taken as presumptive evidence of the sensitivity of these tumors to melanostatin.

TABLE 2. Intensity of Fluorescence of FlNa in Melanoma and Organ Cells

Group	Mel-1	Liver	Spleen	Kidney	Lung
Control	0,530	0,100	0,110	0,300	0,200
Experimental	0,270*	0,078	0,080	0,081*	0,050*

TABLE 3. Intensity of Fluorescence of FlNa in Tumor Cells in Vitro

Group	Mel-5	CLi	LC-1
Control	0,820	0,280	0,167
Experimental	0,515*	0,205*	0,104*

The change in the degree of accumulation of FlNa in the tumors after exposure to melanostatin compared with the control, both in vivo and in vitro, suggest that fluorescent probes can be used to evaluate a chemotherapeutic effect; the difference in fluorescence of different organs can be taken into consideration when the pharmacokinetics of drugs is to be determined. Therapeutic preparations with fluorescent properties can also evidently be used as probes.

The principle of evaluation of the action of chemotherapy described above will be applied in future research.

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