EFFECT OF EPIDERMAL CHALONES ON GROWTH OF TRANSPLANTABLE TUMORS

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An alcoholic extract obtained from rat skin contains epidermal g_1- and g_2-chalones, and when added to a suspension of cells of a transplantable squamous-cell keratinizing carcinoma of the mouse cervix uteri it inhibits its growth by 72.6% when injected into recipient mice. The extract had no inhibitory action on transplantable mouse tumors of other histogenesis (hepatoma 22a, leukemia L-1210, and sarcoma 180). Epidermal chalones had only weak action (inhibiting growth by 39.2%; $P > 0.05$) on an anaplastic transplantable mouse skin carcinoma, which had lost its primary squamous-cell structure in the course of prolonged passage (more than 10 years).

KEY WORDS: *epidermal chalones; inhibition of tumor growth; transplantable tumors of mice.*

Investigation of the possibility of using endogenous tissue-specific inhibitors of cell proliferation (chalones) to inhibit tumor growth is evidently one of the most interesting and important aspects of the study of these substances. Experimental attempts along these lines have been carried out on different models [6, 11, 13]. However, whereas the tissue-specificity of the biological action of chalones has been clearly demonstrated in relation to normal tissues, as regards tumors, which do not differ so greatly in their phenotypical characters because of their low level of differentiation, no such data are available. There are likewise no data on the sensitivity of tumors of the same histogenesis, but at different stages of progression, to the action of chalones, and this is very important from the practical point of view.

In this investigation the action of epidermal chalones on growth of two strains of transplantable tumors derived from epithelium of epidermal type, and three strains of tumors arising from other tissues, was investigated.

EXPERIMENTAL METHOD

Epidermal chalones obtained by alcoholic extraction from rat skin [4, 9] were used. The extract contained two inhibitors of proliferative activity of epithelium of epidermal type. One of them blocked the entry of cells into DNA synthesis by 60-70%, whereas the other blocked the entry of the cells into mitosis by 55-65% (g_1- and g_2-chalones respectively) [14]. A 1% solution of this extract in physiological saline was added to minced tumor tissue at the rate of 1 ml to 100 mg tissue. In the control groups, physiological saline or a 1% solution of bovine serum albumin was added to the tumor tissue in the proportion of 1 ml to 100 mg tumor tissue. Experiments were carried out on the following strains of tumors, maintained in mice: SCC (13th and 14th generations of squamous-cell carcinoma of the cervix uteri, induced by application of a 0.1% solution of DMBA in diethyleneglycol by Vol'fson's method [1]), PRK (squamous-cell carcinoma of the skin, 154th generation [3]), hepatoma 22a, sarcoma 180, and leukemia L-1210. The carefully mixed suspensions of tumor cells in corresponding solutions were injected subcutaneously in a dose of 0.2 ml. Leukemia L-1210 was transplanted intraperitoneally diluted 1:60 with ascites fluid, with the same relative concentration of chalones as for solid tumors. Each animal received an injection of 0.25 ml of the suspension of leukemic cells. Animals with solid tumors were killed 2 weeks after transplantation. The tumor tissue was shelled out, weighed, and the percentage inhibition of tumor growth was deter-

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TABLE 1. Effect of Chalone-Containing Extract (CCE) of Rat Skin on Growth of Transplanted Tumors in Mice

Strain of mice	Strain of tumor	Substance added to physiological saline	Number of mice	Mean weight of tumor, mg	Inhibition of tumor growth, %	P
BALB/c	SCC 13th generation	Bovine serum albumin	15	1069±244	—	
		CCE	15	293±74	72,6	<0,01
	14-th	—	10	1250±253	—	
		CCE	11	378±71	69,8	<0,01
CC57W	PRK	—	16	697±116	—	
		CCE	14	424±129	39,2	>0,05
C3HA	Hepatoma 22a	—	10	769±183	—	
		CCE	10	576±110	25,1	>0,05
C57BL/6	Sarcoma 180	—	9	690±117	—	
		CCE	11	790±143	14,5	>0,05

mined. The action of epidermal chalones on L-1210 was assessed by the method described by Sof'ina [7]. The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The results, summarized in Table 1, revealed differences in the sensitivity of the tumors used to the epidermal chalones. Distinct inhibition of tumor growth by the chalone was detected in the case of squamous-cell carcinoma of the cervix uteri (strain SCC), about equally for tumors of the 13th and 14th generations. Since the protein components of the alcoholic fraction of the rat skin extract are foreign for mice and could act nonspecifically on growth of the transplanted tumor, the effect of adding another foreign protein — bovine serum albumin — to the suspension of tumor cells was investigated. It was found that this did not affect growth of strain SCC. The effect of epidermal chalones on squamous-cell carcinoma of the skin (strain PRK) was not significant ($P > 0.05$). A histological study of the generation of PRK used showed that it had lost its primary squamous-cell structure and, on the basis of its morphological features, it could be identified as a cytoblastoma, i.e., an anaplastic tumor of epithelial nature, no longer with the morphological features of the original tissue. The epidermal chalones had no inhibitory action on growth of tumors of different histogenesis (mesodermal and entodermal) (Table 1). The epidermal chalones did not affect the life span of $(C57BL/6 \times DBA/A2)F_1$ mice with transplantable leukemia L-1210 (control (7.8 ± 0.4 days, after injection of the chalone-containing extract 8.1 ± 0.1).

The well-known basic characteristic of the chalones — their tissue-specific inhibitory action on cellular prolieration — thus extends not only to normal tissue, but also to tumors, a matter of great practical importance. The writers recently showed that epidermal chalones are tissue-specific also in their antigenic structure [5], and this greatly widens the possibilities of their study and use. In previous experiments on mice, the preparation of epidermal chalones used exhibited marked biological activity in a sessional dose as low as 1.5 mg [2]. However, considering the observed decrease in the sensitivity of tumor cells to chalones [10, 14], and also the fact that the squamous-cell tumor used originated from the cervico-vaginal epithelium, which under normal conditions may be less sensitive to them than the epithelium of the skin, in this investigation sufficiently large doses of chalones were used. Nevertheless, their effect was weaker against strain PRK, originating from the epithelium of the skin, the most sensitive structure to epidermal chalones. The writers are inclined to explain this fact by differences in the degree of differentiation of these strains and, in particular, the marked anaplasia of the PRK tumor. It can tentatively be suggested that the sensitivity of the tumor cells to the chalones of normal tissues is not constant and perhaps diminishes during progression of the tumor. The study of the qualitative and quantitative aspects of the mechanisms of this phenomenon during carcinogenesis and during progression of the tumor process is, in the writers' opinion, interesting on its own account.

The results described above confirm the justification for the experimental study of the use of chalones for tissue-specific treatment of neoplasms. This may take the form not only of their administration independently (although this possibility is not ruled out on theoretical grounds [8, 12]), but also the use of chalones as tissue-specific cytostatics as a sup-

porting measure during the pharmacotherapy, radiotherapy, or immunotherapy of tumors.

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HOMOLOGOUS ANTITISSUE ANTIBODIES AS A FACTOR INHIBITING THE DEVELOPMENT OF MALIGNANT TUMORS

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Experiments with transplanted and induced tumors showed that the physiological system of autoimmunity (homologous antitissue antibodies) can be used to increase resistance of the host to growth of malignant neoplasms. A method is developed for obtaining a globulin preparation containing normal antitissue antibodies in high titer.

KEY WORDS: *resistance; autoimmunity; normal antibodies; carcinogen.*

The attention of research workers is at present being increasingly drawn toward the use of natural immunological mechanisms of defense against the appearance and growth of malignant neoplasms. The use of normal antitissue antibodies, concerned in the regulation of metabolic processes, the removal of products of tissue metabolism and tissue breakdown, and also performing other no less important physiological functions, can be emphasized in this respect [1, 2, 6-8, 11-13].

During growth of a malignant neoplasm continuous autoimmunization of the host takes place under the influence of breakdown products arising from the tumor. In Klemparskaya's opinion, autoimmunization inhibits the response to the specific tumor antigen and may be one of the causes preventing rejection of the tumor.

The immunodepressive effect of tissue autoallergy is well known [6]. One way of suppressing autoallergy is by injecting ready-made preparations of normal antitissue antibodies.

The object of this investigation was to develop a new experimental method of increasing resistance of the host to growth of a malignant neoplasm by injection of special prepara-

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