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ACTION OF EPIDERMAL CHALONES ON PROLIFERATION AND DIFFERENTIATION OF MOUSE LOWER LIP TUMOR CELLS

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The discovery of tissue-specific regulators of cell proliferation, or chalones [9], has raised the urgent question of their use as tumor inhibitors. The first research in this direction has already been undertaken and some hopeful results obtained [3, 6, 10].

The object of the present investigation was to study the effect of epidermal chalones (EC) on the onset and development of lower lip tumors induced in mice by methylcholanthrene (MCh).

EXPERIMENTAL METHOD

Altogether 110 male CC57BR mice, divided into five groups, were used. A 0.5% solution of MCh in acetone was applied 3 times a week for 2 months to the mucosa of the lower lip of all the animals. The mice of group 1 received no other treatment. The mice of groups 2 and 4 received a preparation of EC consisting of an alcoholic extract of the epidermis of rat skin. The method of isolation of the extract and its activity, reflecting the presence of epidermal G_1- and G_2-chalones, was described previously [4]. The choice of method of administration of the chalone was based on data showing that systemic administration of chalone-containing extracts can cause death of animals [8], whereas local application is safe [6]. The EC extract was infiltrated into the tissues of the lower lip in a dose of 100 μg in 0.1 ml of 0.9% NaCl. This dose proved effective previously in inhibiting carcinogenesis in mice [6].

To study the effect of EC on the onset of tumors, the extract was injected into the mice of group 2 1 h after each application of MCh, and thereafter 3 times a week for 1 month after the end of MCh application. To investigate the effect of EC on neoplasms already formed, EC began to be given to the mice of group 4 1 week after the end of MCh application, when 50% of the mice had developed a neoplasm of the lower lip. In that case the EC preparation was injected 3 times a week for 3 months. As controls, the mice of groups 3 and 5 received a similar ethanol extract of liver containing G_1- and G_2-hepatic chalones (HC), in the same way and in the same dose as the animals of groups 2 and 4 respectively [5].

EXPERIMENTAL RESULTS

Application of MCh induced the appearance of multiple papillomas and of carcinoma of the mucosa and skin of the lower lip in all mice. The degree of keratinization was estimated in accordance with the known classifications [1, 2].

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TABLE 1. Effect of Chalones on Development of Lower Lip Tumors in Mice

Group	No. of mice in group	Life span, days	Time of onset of first tumors, days	Time from discovery of first tumors until death, days	No. of tumors per mouse	No. of regressing tumors per mouse	
						absolute	%
1 (MCh)	24	164±4,7	122±5,2	41±3,6	5,0±0,25	—	—
2 (EC + MCh)	24	192±9,2*,‡	116±5,9	76±8,1*,‡	3,0±0,39	1,6*,‡	53
3 (HC + MCh)	22	141±7,2	109±7,8	39±5,5	3,8±0,45	0,1	3
4 (MCh + EC)	20	195±6,1*	125±6,9	64±4,0‡	4,1±0,36	2,3†,‡	56
5 (MCh + HC)	20	179±6,2	126±3,9	54±5,8	3,3±0,34	0,2	6

*P < 0.01 compared with group 3.
†P < 0.01 compared with group 5.
‡P < 0.01 compared with group 1.

TABLE 2. Microscopic Characteristics of Lower Lip Tumors in Mice in Experiments with Application of Methylcholanthrene and Injection of Chalones

Group	No. of mice														
	in group	with papillomas		with squamous cell carcinoma								depth of invasion			
				I		II		III		IV		to muscles		whole lip	
		abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
1- (MCh)	24	2	8,3	6	25,0	8	33,3	5	20,8	3	12,6	8	36,4	14	63,6
2- (EC+MCh)	24	4	16,7	14	58,3	4	16,6	1	4,2	1	4,2	16	80,0	4	20,0
3- (HC+MCh)	22	2	9,1	6	27,3	2	9,1	8	36,3	4	18,2	5	25,0	15	75,0
4- (MCh+EC)	20	1	5,0	10	50,0	5	25,0	2	10,0	2	10,0	15	79,0	4	21,0
5- (MCh+HC)	20	2	10,0	5	25,0	6	30,0	2	10,0	5	25,0	6	33,0	12	67,0

Legend. Degree of keratinization. I) Highly differentiated carcinoma, II) moderately differentiated carcinoma, III) undifferentiated keratinizing, IV) non-keratinizing carcinoma.

It will be clear from Table 1 that administration of EC simultaneously with MCh caused a significant increase in the life span of the animals compared with mice receiving HC and with the controls without chalones. A characteristic feature was a marked lengthening of the period from the first discovery of tumors in the animals of group 2 until their death, evidence of slowing of proliferation and growth of the neoplasms. The duration of survival after discovery of tumors in mice receiving EC in the period of tumor formation and growth (group 4) was found to be increased compared with the control. The volume of the tumors at the same times of the experiments was always less in animals receiving EC than in animals receiving HC. These results can be summed up in the statement that EC inhibited growth of neoplasms which had already arisen. The frequency of regression of tumors in mice receiving EC, incidentally, was higher than in the group receiving HC.

Microscopic investigation (Table 2) revealed that injection of EC led to an increase in the degree of differentiation of the cells and to a decrease in the depth of invasion. The increase in the degree of keratinization was perhaps one cause of the more frequent regression of tumors in mice receiving EC, and inhibition of invasive growth led to an increase in the survival period of the animals.

Following administration of EC at different stages of carcinogenesis, development of the tumors was thus inhibited, for which a decrease in the rate of cell proliferation and stimulation of differentiation, which is also regarded as one mechanism of action of the chalones [7], were responsible. In the present experiments inhibition of proliferation of normal cells reduced their sensitivity to carcinogenic action, whereas inhibition of proliferation of cells transformed by the carcinogen delayed growth of the neoplasms. The results indicate that the further study of the possible clinical use of chalones as inhibitors of tumor growth may be promising.

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