EFFECT OF EPIDERMAL CHALONES ON INDUCTION AND GROWTH OF TUMORS OF THE CERVICOVAGINAL EPITHELIUM IN MICE

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During induction of tumors of the cervicovaginal epithelium by means of dimethylbenzanthracene in female BALB/c mice or after their appearance, a 1% solution of chalones contained in 55-81% ethanol extracts of rat skin or liver (control) was applied intravaginally. The use of skin extract during induction of tumors, but not the use of liver extract, was shown to retard growth of tumors of the vagina and cervix uteri a little and to reduce the frequency of their appearance; as a result of their action on neoplasms already formed in this situation the chalones prolonged the survival of the animals with tumors.

KEW WORDS: chalones; cervicovaginal epithelium; tumor of the cervicovaginal epithelium; 7,12-dimethylbenz(a)anthracene.

Attempts to establish an experimental basis for theuse of chalones in the treatment of malignant neoplasms have so far been restricted to the study of their ability to inhibit growth of transplantable tumors [8-11], and there are no data on the effect of chalones on induced tumors. This is partly because of the difficulty of such experiments because of inability of animals to tolerate prolonged systematic administration of chalone-containing extracts [12,13]. For this reason the local application of chalones to inhibit induction and progression of tumors is of practical interest.

The writers showed previously that transplantable tumors of the cervicovaginal epithelium of mice are sensitive to the inhibitory action of epidermal chalones contained in a lyophilized alcoholic extract of rat skin [3, 4, 14].

In the present investigation the effect of epidermal chalones was studied on induction and growth of tumors in this situation.

EXPERIMENTAL METHOD

Experiments were carried out on 92 female BALB/c mice aged 3 months, obtained from the "Rappolovo" nursery, Academy of Medical Sciences of the USSR. There were three series of experiments. In series I on 35 mice, a polyurethane sponge soaked in a 0.1% solution of 7,12-dimethylbenz(a)anthracene (DMBA, from Fluka-Busch, Switzerland) in diethylene glycol was introduced into the vaginal cavity twice a week for 2 months, by Vol'fson's method [2]. The sponge was removed 1 h after application of the carcinogen and was replaced by a similar sponge, but soaked in a 1% solution of chalones, contained in extracts of rat skin and liver (control) in 0.9% NaCl solution. The solutions were made up when required from lyophilized ethanol extracts of the corresponding tissues [3, 5, 6], and the sponges soaked in them were removed immediately before the next application of the carcinogen. All the mice of this series were killed on the 70th day of the experiment.

In the experiments of series II sponges with the carcinogen were introduced into 32 mice 3 times with an interval of 3 days, after which sponges soaked in solutions of epidermal or hepatic chalones (11 animals in each group) or with 0.9% NaCl solution (10 animals) were introduced into the vaginal cavity of the mice twice a week for 2 months. All the mice of this group were killed on the 64th day of the experiment.

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TABLE 1. Effect of Chalone-Containing Extracts of Skin and Liver on the Frequency of Appearance and Histological Structure of Cervicovaginal Tumors Induced by DMBA in BALB/c Mice

Item	Series of experiments						
	I		II			III	
	EC	HC	EC	HC	0.9% NaCl solution	EC	HC
Number of mice	15	20	11	11	10	12	13
Number of mice with tumors	15	20	7	10	9	11	13
Mean latent period of tumor, days	48±0,37	44,8±0,54	—	—	—	—	—
Mean length of survival of mice, days	—	—	—	—	—	207±17,8	169±11,3
Histological type of tumor:							
carcinoma in situ	1	—	2	5	3	—	—
squamous-cell carcinoma	11	15	4	3	4	7	4
squamous-cell carcinoma with keratini.	1	3	1	2	2	3	8
adenosquamous carcinoma	2	2	—	—	—	1	1

Legend. EC) Administration of epidermal chalone-containing extract; HC) administration of hepatic chalone-containing extract.

In the experiments of series III on 25 mice, a sponge soaked in DMBA solution was introduced into the vaginal cavity twice a week for 2 months. After the end of applications of the carcinogens a sponge soaked in a solution of epidermal or hepatic chalones (12 or 13 mice respectively) was introduced into the vagina twice a week until the end of the experiment. The surviving mice in this series of experiments were killed on the 300th day. Just as in the experiments of series II, the sponges soaked in solutions of the chalones were removed immediately before the next insertion of the fresh compound.

In the course of the experiment the state of the vaginal mucosa was assessed visually, and after the end of the experiment total preparations of the vagina and cervix uteri of mice of all series were examined microscopically.

EXPERIMENTAL RESULTS

Previous experiments showed that 55-81% of ethanol extracts of rat skin and liver contain nonspecific impurities as well as G_1- and G_2-chalones of the corresponding tissues [3, 5, 6]. For that reason the parallel use of the liver extract served not only as control of the tissue-specificity of the inhibitory action of the epidermal chalones on the squamous-cell epithelium of the vagina and cervix uteri, but also as a control of the possible nonspecific effect of impurities contained in the skin extract on turmor growth.

The experimental results are summarized in Table 1. The experiments of series I showed that epidermal chalones retarded the appearance of visually observable neoplasms a little. However, it should be remembered that this assessment was largely subjective and, in some cases, in the absence of visible tumors they could be shown to be present histologically. What was observed here was thus not so much the effect of epidermal chalones on latent period as inhibition of the rate of growth of induced neoplasms of the cervicovaginal epithelium.

When the epidermal chalones were applied immediately after short exposure to the carcinogen (series II) their effect was manifested by the fact that tumors developed in seven of the 11 mice (64%), whereas after application of hepatic chalones or of the solvent alone they developed in 91% and 90% of cases respectively.

From the practical point of view, the results of the experiments of series II, in which chalones were applied to mice in which tumors were already developing, are of the greatest interest. As Table 1 shows, the length of survival of mice receiving intravaginal application of epidermal chalones was increased. It should be emphasized that by the end of the experiment (the 300th day) four of the 12 mice were still alive in this group, whereas the last control animal had died on the 236th day. Moreover, in mice treated with epidermal chalones, the neoplasms were somewhat smaller in size (tumors over 0.5 cm in diameter were found in three of 12 such mice compared with eight of 13 control animals). Another indirect indicator of the inhibitor action of epidermal chalones on growth and progression of tumors from target tissues is the fact that under the influence of these chalones squamous-cell keratinizing

carcinomas were found less frequently than under the influence of hepatic chalones, which are not specific for the cervicovaginal epithelium (Table 1).

The inhibitory effect of epidermal chalones on growth and progression of induced tumors of the vagina and cervix uteri in mice was thus demonstrated for the first time in this investigation. Despite the fact that this effect was only of the nature of a tendency, the writers find the results encouraging from both the prophylactic and the therapeutic points of view, more especially because tumors in this location are very common and the results of their treatment are still unsatisfactory [1]. Perhaps the therapeutic application of highly purified preparations of chalones, instead of crude tissue extracts, would result in a more selectively oriented effect of these tissue-specific inhibitors on tumor growth.

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