INVESTIGATION OF THE CYTOSTATIC ACTION OF CYCLOPHOSPHAMIDE ON ESOPHAGEAL TUMORS AND EPITHELIUM IN MICE

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The cytostatic action of cyclophosphamide on sarcoma 37 and the esophageal epithelium was investigated in albino mice by determination of the diurnal rhythms of mitotic activity and the number of labeled nuclei. Tumor cells were shown to be most sensitive to the inhibitory action of the compound evidently in the G_1 phase at the beginning of the S phase. As the period of DNA synthesis came to an end, the resistance of the cells to the cytostatic increased. Cells in the G_1 phase of the mitotic cycle in the esophageal epithelium are sensitive to the inhibitory action of cyclophosphamide.

KEY WORDS: mitotic cycle; cyclophosphamide; sarcoma 37; esophageal epithelium.

If certain antitumor preparations are administered at different times of the 24-h period their cytostatic action both in normal [4] and tumor [1, 2, 6] tissues has been found to vary. This can be explained by diurnal fluctuations in the number of cells in a given phase of the mitotic cycle and the preferential action of the corresponding preparations on certain of these phases [4]. In the present investigation the inhibitory action of cyclophosphamide on cellular proliferation of sarcoma 37 (solid form) and the esophageal epithelium was investigated in mice by correlation with diurnal rhythms of mitotic indices and indices of labeled nuclei.

EXPERIMENTAL METHOD

Experiments were carried out on 120 noninbred male albino mice weighing 30 g kept under conditions of natural lighting and free access to food. All the animals were inoculated with the tumor. Ascites fluid containing sarcoma 37 cells was diluted with sterile physiological saline and injected subcutaneously into the axillary region of the animal in a dose of 0.1 ml. The experiments were carried out on the 9th day after inoculation. Cellular proliferation was inhibited by cyclophosphamide, which has an alkylating action. This action disturbs processes taking place in the S phase [5] and also in the G_2 phase [8]. Expecting on the basis of previous data [3] that the number of cells in these phases would be maximal at 1-4 p.m., the cytostatic was injected at 2 p.m. The cyclophosphamide was diluted before use in sterile isotonic sodium chloride solution and injected intraperitoneally in a dose of 80 mg/kg. The first batch of animals, treated with cyclophosphamide, was sacrificed at 3 p.m. (1 h after injection of the cyostatic), later batches at 2-hour intervals from 4 p.m. to 10 a.m. next day (12th sacrifice). Control mice were killed at 2-hour intervals over a period of 24 h. All the animals were given [^{3}H]thymidine in a dose of 30 μCi (specific activity 16 Ci/g) intraperitoneally 1 h before sacrifice. Sections cut to a thickness of 5 μ were coated with type M photographic emulsion, exposed for 10-30 days, and stained with Carrazzi's hematoxylin. Nuclei above which there were five or more grains of silver were regarded as labeled. The mitotic indices (MI) and indices of labeled nuclei (ILN) were expressed in promille, after examination of 6000-9000 tumor cells and 9000-10,000 cells of the basal layer of the epithelium in the distal third of the esophagus in each case. Sections through the tumor nodule were investigated over the whole area from the capsule to the central (usually necrotic) zone. The results (for at least five animals at each time of the investigation) were subjected to statistical analysis by the Fisher–Student method. Differences for which $P \leq 0.05$ were considered to be significant.

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TABLE 1. Changes in MI and ILN (in ‰) in Sarcoma 37 and Esophageal Epithelium in Mice Treated with Cyclophosphamide

Time of day	Esophagus				Tumor			
	control		injection of cyclophosphamide at 2 p.m.		control		injection of cyclophosamide at 2 p.m.	
	MI	ILN	MI	ILN	MI	ILN	MI	ILN
15			2,6*	9,4*			7,8*	21,9*
16	3,9	27,7	2,2	21,8	12,0	167,5	6,5	37,7
18	2,9	5,4	1,8	9,6	11,3	74,3	5,1	5,9
20	1,2	30,0	1,6	1,7	11,5	91,0	2,4	22,7
22	2,4	83,2	2,0	10,3	14,0	85,3	2,9	11,6
24	1,1	71,4	0,5	36,7	13,1	27,9	0,8	11,1
2	1,8	35,5	0,6	18,5	10,2	6,5	3,5	0,8
4	6,8*	116,2*	1,3	6,8	7,4*	62,4*	2,6	0,1
6	11,4	172,1	3,0	39,7	10,9	47,4	2,8	1,7
8	8,6	58,6	4,2	71,3	10,4	111,0	4,5	3,4
10	8,6	93,9	2,7	107,1	7,9	180,0	2,8	6,8
12	6,5	40,0	7,0	57,6	8,5	74,7	6,3	44,5
14	5,3	26,0			10,8	35,2		
Mean	5,0	63,3	2,5	32,5	10,7	80,1	4,0	14,0

* First sacrifice.

EXPERIMENTAL RESULTS

The study of the action of cyclophosphamide on the esophagus showed that cells in the G_1 phase of the mitotic cycle were probably sensitive to the compound. The cytostatic activity of mammalian (mouse, rat, dog) serum becomes maximal 15 min after injection of cyclophosphamide in doses of 10 to 1000 mg/kg, after 2 h it is reduced by 50%, and after 24 h it cannot be detected [7]. Even so, the values of MI in the experimental series did not differ significantly from those in the control during the 14 h after injection of the compound (Table 1). Only later, from 4 to 10 a.m., was the mean MI for the experimental animals (2.8‰) reduced to one third of the control value (8.9‰; P = 0.002). These results indicate that the G_2 phase of mitosis (in the period of maximal cytostatic activity the cells commenced mitosis without hindrance) and, evidently, in the period of DNA synthesis (processes preceding mitosis by 14 h were found to be sensitive to cyclophosphamide) are insensitive to cyclophosphamide. Changes in ILN indicate that cells in the S phase are resistant to the inhibitory effect of cyclophosphamide. It will be clear from Table 1 that maximal differences in the values of ILN in the control and experimental animals did not begin to appear until 6 h after injection of cyclophosphamide. For instance, in the interval from 8 p.m. to 6 a.m. the mean ILN for the experimental animals (18.9‰) was only 22% of its value in the control animals (85.0‰; P = 0.016). Sensitivity of the G_1 period to cyclophosphamide can be judged on the basis of the following fact. In the control animals the 6-hour maximum of MI (P_{6-8} = 0.0001, P_{6-2} = 0.0001), indicating the approximate beginning of the G_1 period for the largest number of naturally synchronized cells, was observed 8 h before injection of cyclophosphamide (2 p.m.), and the beginning of the rise in ILN (after 8 p.m.), roughly indicating completion of the G_1 period by the group of naturally synchronized cells, was observed 6 h after injection of the compound.

In the tumor, analysis of proliferative activity in the control animals revealed at least two cell populations. For instance, the absence of a peak of MI in the period between the 10 a.m. and 4 p.m. peaks of ILN (P_{10-14} = 0.022, P_{10-12} = 0.002, P_{14-16} = 0.044, P_{16-2} = 0.016) suggests that the maxima of ILN characterized the processes of DNA synthesis not in two consecutive mitotic cycles, but in two cell populations. The action of cyclophosphamide was seen to be strongest on the tumor cells that were evidently in the G_1 period and the beginning of the S phase, and it weakened gradually toward the end of the period of DNA synthesis. The resistance of cells in the G_2 phase and in mitosis indicates that MI of the experimental animals differed from the controls only 4 h after injection of the cyclostatic, i.e., after 6 p.m. (P = 0.023). The gradual increase in these differences up to a maximum in the course of 6 h (from 6 p.m. to midnight) and their manifestation 4-10 h after injection of cyclophosphamide point to a gradient of the inhibitory action of the compound on cells most probably in the S phase. It is evidence that the lower value of MI than in the control in the later stages (from midnight to 10 a.m.) was connected with the inhibitory effect of cyclophosphamide on cells in the G_1 period of the mitotic cycle. The low level of ILN throughout the 24-h period in the experimental animals (the mean value of ILN in the experimental series (14.0‰), which was only 18% of the control value (P = 0.0001) can be completely explained by the effect of cyclophosphamide on cells in the G_1 and S phases of the mitotic cycle. The view is now put forward on the point of application of the inhibitory action of cyclophosphamide are not contradicted by data

obtained on mice with hemocytoblastosis La [2] and also with carcinoma of the forestomach [1]. Cells of the latter, incidentally, are sensitive to the action of cyclophosphamide when in the G_2 phase also.

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EFFECT OF SINGLE AND REPEATED PREGNANCY ON FREQUENCY OF ORIGIN OF PRIMARY TUMORS INDUCED BY SV_{40} VIRUS IN SYRIAN HAMSTERS

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The effect of single and repeated pregnancy on the frequency of origin of primary tumors induced by SV_{40} virus was investigated in Syrian hamsters. Females developed tumors after 1 to 5 pregnancies significantly less frequently during the latent period of SV_{40} carcinogenesis than females not becoming pregnant in the same experiment. However, these differences are evidently not attributable to immunization of the gravid females by embryonic antigens, for the frequency and times of origin of primary tumors in males were the same as in previously gravid females.

KEY WORDS: pregnancy; SV_{40} virus; embryonic antigens.

The possibility of using embryonic antigens for antitumor immunization has been widely discussed in recent years. The idea is based on somewhat contradictory evidence. For instance, immunization with embryonic cells taken at certain early stages of pregnancy prevented the appearance of primary tumors and growth of transplantable tumors [1, 2]. According to other workers, repeated pregnancy during the latent period of carcinogenesis led to a decrease in the incidence of tumors in previously pregnant females compared with virgins, an effect interpreted as evidence of immunization of the mother by embryonic antigens of the fetus [3]. However, some of the published data has not been confirmed by other workers [4, 5] and, on the whole, the question of whether embryonic antigens can be used for antitumor immunization still remains undecided.

The object of this investigation was to study the effect of single and repeated pregnancy on the incidence of primary tumors induced by SV_{40} virus in Syrian hamsters.

EXPERIMENTAL METHOD

Noninbred Syrian hamsters were obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR. The experiments were so designed that in females infected with SV_{40} virus on the first day of life the latent period of carcinogenesis coincided with the first to fifth pregnancy. The control to this experiment

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