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DIURNAL RHYTHMS OF CELL PROLIFERATION IN LATE PRECANCEROUS CHANGES INDUCED IN THE LIVER BY ORTHOAMINOAZOTOLUENE

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Orthoaminoazotoluene was administered to mice for 9 months. Diurnal rhythms of mitotic activity and of the number of DNA-synthesizing cells were established by autoradiography with ^{3}H-thymidine in stages II (adenomatous nodules) and III (primary hepatomas) of carcinogenesis in the developing tumors and surrounding parenchyma of the liver. A monomodal rhythm of mitotic activity was demonstrated in the structures mentioned above, with the number of mitoses reaching a maximum at 4-7 a.m., whereas the diurnal rhythm of the index of labeled nuclei was bimodal, with maxima at 7 p.m. and 4 a.m. The mean diurnal values of both indices at stages II and III of hepatocarcinogenesis were considerably higher than in the surrounding noncancerous liver tissue.

KEY WORDS: orthoaminoazotoluene; late stages of hepatocarcinogenesis; diurnal rhythms of mitosis and of DNA-synthesizing cells.

An important addition to Founds' concept of tumor progression is represented by the successive morphological stages of precancerous changes in organs and tissues common to different types of carcinogenesis, established by Shabad [9].

There is every reason to suppose that growth which has escaped to some degree from the control of the organism is one of the first stages of progression and is an inseparable property of any tumor [8].

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TABLE 1. Diurnal Changes in MI and ILN (in %) of Cells of Primary Hepatomas, of Adenomatous Nodules, and of Surrounding Noncancerous Liver Tissue of Mice Receiving OAAT for 9 Months

Time of day	Primary hepatomas				Adenomatous nodules				Noncancerous liver tissue			
	MI	P	ILN	P	MI	P	ILN	P	MI	P	ILN	P
10	1,38±0,14		6,31±0,64		0,82±0,26		3,96±0,97		0,19±0,08		1,34±0,17	
13	1,10±0,05	0,469	8,80±0,40	0,093	0,33±0,07	0,095	5,83±0,45	0,068	0,08±0,02	0,235	1,98±0,18	—
16	0,70±0,40	0,057	6,60±1,80	0,353	0,25±0,04	—	6,75±0,85	—	0,07±0,02	—	2,21±0,23	0,078
19	0,86±0,19	—	15,2±1,37	0,019	0,28±0,05	—	9,80±1,34	0,080	0,08±0,03	—	2,80±0,50	—
22	0,80±0,16	—	13,5±4,34	0,428	0,20±0,03	—	8,66±0,85	—	0,03±0,05	—	2,43±0,26	—
1	1,51±0,88	0,591	2,10±0,20	0,039	0,27±0,06	—	1,70±0,16	0,001	0,06±0,02	—	0,77±0,17	0,001
4	2,80±1,24	0,452	13,1±5,13	0,161	0,59±0,09	0,039	6,21±0,74	0,001	0,09±0,07	—	2,14±0,22	0,001
7	1,30±0,25	0,235	11,1±1,30	0,325	1,34±0,36	0,161	4,81±0,36	0,136	0,34±0,11	0,003	1,29±0,16	0,008
Mean values for 24 h	1,31±0,30	$P_{16,19,22-1}=0,019$ $P_{4-10,13}=0,009$	9,60±0,95	$P_{19-1}=0,001$ $P_{1-4,7}=0,019$ $P_{10-7}=0,001$	0,51±0,12	$P_{16-7}=0,013$ $P_{1-7}=0,01$	5,98±0,93	$P_{10-19}=0,006$ $P_{19-1}=0,001$ $P_{10-4}=0,028$	0,12±0,03	$P_{7-13}=0,001$	1,86±0,25	$P_{10-19}=0,004$

The main ways leading to tumor growth are disturbances of regulation of cell multiplication and differentiation [7, 8]. Diurnal rhythms of dividing and DNA-synthesizing cells, characteristic both of normal tissues [1, 4, 5] and of many transplantable tumors [3, 12, 13], are one of the most important criteria by which the principles of cell proliferation can be judged. This suggests that the study of the principles governing the rhythmic character of cell multiplication during the 24-hour period in the various stages of progression typical of carcinogenesis [7, 9, 10] would be interesting as a way of studying the sensitivity of transformed target cells to various factors concerned in the mechanism of the diurnal rhythm of cell division.

The object of this investigation was to study the diurnal rhythm of mitotic activity and of the number of DNA-synthesizing cells in the late stages of precancerous changes of induced carcinogenesis of the liver.

EXPERIMENTAL METHOD

Noninbred male mice with a mean weight of 20-25 g were used. The animals received orthoaminoazotoluene (OAAT) by direct injection into the esophagus from a syringe with a blunt curved needle, in the form of a 1% oily solution in a dose of 0.1 ml per mouse three times a week for 9 months (the total dose was 120 mg per mouse). The animals were given a natural diet. Mice which survived 10 months (1 month after the end of OAAT administration) were killed in groups (of 12 or 13) at intervals of 3 h during the 24-h period. ^{3}H-Thymidine (USSR) with an activity of 19.8 Ci/mmole was injected 1 h before sacrifice in a dose of 0.5 μCi/g body weight. Pieces of liver and of the tumors were fixed in Carnoy's solution. Sections 5 μ thick were coated with type M emulsion. The exposure lasted 30 days. Autoradiographs were stained with Carazzi's hematoxylin. Cells containing more than three grains of silver above the nucleus were regarded as labeled.

The proliferative activity of cells forming adenomatous nodules, of the hepatocytes surrounding them, and also of the primary hepatomas, was investigated in the liver of the experimental mice. Because of the pattern of the morphological structure it was impossible to draw a sharp line between an adenoma arising from parenchymatous cells and a trabecular hepatoma [2]. A conventional boundary was accordingly drawn between adenomatous nodules and tumors, nodes visible macroscopically and having their own vascular wall being regarded as primary tumors [2].

The mitotic index (MI) and index of labeled nuclei (ILN) were determined in promille, by counting 10,000-20,000 cells separately for each type of tissue studied in each mouse.

EXPERIMENTAL RESULTS

Histological study of the liver of mice which survived 10 months from the beginning of the experiment showed complete disruption of the structural pattern of the liver parenchyma with highly polymorphic foci of proliferating epithelium of the biliary passages, separated by proliferating "oval cells," frequently accompanied by degenerating hepatocytes. Foci of nodular hyperplasia of hepatocytes arranged chaotically or forming structures resembling trabeculae, were seen. Among the primary tumors, found in 41% of the experimental mice surviving 10 months, only neoplasms of hepatocellular histogenesis were studied (mainly travecular hepatomas, less frequently anaplastic carcinomas). Data on cell division are given in Table 1. They show that

the rhythm of ILN of cells of the adenomatous nodules had two maxima during the 24-h period. The main maximum of ILN was observed at 7 p.m. and the smaller maximum at 4 a.m. The minimal values of ILN occurred at 10 a.m. and 1 a.m. Diurnal changes in MI in the same tissue had a well-marked monophasic character with high values of MI at 7 a.m. and minimal values at 4-10 p.m. A significant increase in the number of mitoses, which was found at 7 a.m., occurred 12 h after the first maximum of ILN and 3 h after the second.

Similar changes in the dynamics of the dividing and DNA-synthesizing hepatocytes during the course of the 24-h period were observed in the liver parenchyma surrounding the foci of nodular hyperplasia: Maxima of ILN were observed at 7-10 p.m. and 4 a.m. and a maximum of MI at 4 a.m. The interval between the maxima of ILN and MI (12 h for the main and 3 h for the second maximum of ILN) also were similar.

A study of the proliferative activity of the primary liver tumors showed that the diurnal rhythm of DNA-synthesizing cells also was bimodal in character. The largest number of labeled cells was found at 7 p.m., a smaller maximum of ILN was observed at 4 a.m., and minima at 10 a.m.-4 p.m. and at 1 a.m. Diurnal changes in MI in the tumor cells followed a monomodal curve with a maximum of the number of mitoses at 4 a.m. and a minimum at 4-10 p.m. It is interesting to note that a maximum of ILN was observed 9 h before the maximum of MI, although the second maximum of labeled cells coincided in time with the maximum of MI in the early morning.

The curve of the diurnal dynamics of DNA-synthesizing cells of adenomatous nodules and primary hepatomas induced in the liver by OAAT was thus similar and was bimodal in character. In a previous investigation [6] the writer demonstrated a bimodal rhythm of DNA-synthesizing cells of the liver parenchyma in an earlier stage (focal proliferation) of hepatocarcinogenesis. Consequently, the biphasic diurnal rhythm of DNA-synthesizing cells found in the earlier stages of precancerous changes in the liver still persists at the later stages of OAAT-induced carcinogenesis, distinguishing it considerably from the monophasic rhythm characteristic of normal hepatocytes of adult mice [4, 6].

The diurnal rhythm of mitotic activity discovered previously in the earlier stages of carcinogenesis of the liver [6] and also in the later stages investigated in the present experiments was monomodal in character with a maximum of the number of mitoses in the early morning and a minimum in the evening or at night. It was very similar to the diurnal dynamics of mitotic activity observed by other workers in normal hepatocytes of adult mice and rats [1, 5].

The mean diurnal ILN was 9.3 times greater than MI in the hepatomas, 11.9 times greater in the adenomatous nodules, and 15.5 times greater in the surrounding liver tissue. In primary hepatomas and adenomatous nodules higher mean diurnal indices were found for the number of dividing and DNA-synthesizing cells than in the surrounding liver tissue ($P \leq 0.02$), evidence of a much larger number of cells entering the S and M phases of the mitotic cycle during the 24-h period in these structures.

The results thus point to the existence of a diurnal rhythm of mitosis and of DNA-synthesizing cells at the stage of focal proliferation and of primary hepatomas. Similar patterns for processes of cell proliferation have also been found in transplantable hepatomas at later stages of progression [12, 13]. A diurnal rhythm of cell reproduction is thus typical of tumor growth both in the initial and in the later, qualitatively different stages of tumor development.

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CARCINOGENESIS INDUCED BY POLYCYCLIC AROMATIC HYDROCARBONS IN MALE AND FEMALE (CBA × C57/BL)F_1 MICE

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Sex differences were found in the development of skin tumors induced by 3-methylcholanthrene and benz(a)pyrene in (CBA × C57/BL)F_1 mice. Males were more susceptible to the induction of skin tumors than females.
KEY WORDS: carcinogenesis; 3-methylcholanthrene; benz(a)pyrene; sex differences.

Sex differences in the statistics of malignant neoplasms have been found in both clinical and experimental studies [4]. Excluding the reproductive organs, cancer is found much more frequently among men than among women. This applies to malignant neoplasms of the liver, lungs, stomach, rectum, pancreas, urinary bladder, skin, etc. The exceptions are carcinoma of the thyroid gland and melanoma, which are more common in women.

Experimental findings also confirm the presence of sex differences in the development of both spontaneous tumors and tumors induced by chemical carcinogens in hamsters, rats, and mice. There is comparatively little information in the literature on the effect of the sex of experimental animals on the development of cutaneous chemically induced carcinogenesis. For instance, according to Bates [1], male mice are more susceptible to the carcinogenic action of 7,12-dimethylbenzanthracene than females. A similar fact has been observed by other workers studying hamsters [3] and rats [2] following subcutaneous injection of 3-methylcholanthrene.

The object of this investigation was to study whether sex differences occur in the frequency of skin tumors induced by polycyclic aromatic hydrocarbons (PAH) in hybrid (CBA × C57/BL) mice.

EXPERIMENTAL METHOD

Mice aged 2 months were used. The carcinogens were 3-methylcholanthrene (3-MC) in 0.5% solution in benzene and benz(a)pyrene (BP) in a 0.6% solution in benzene. In the experiments of series I 3-MC was applied to a previously shaved area of skin in the interscapular region in a dose of 0.02 ml daily until the appearance of malignant tumors. In the experiments of series II 3-MC was applied once in the same dose followed 2 weeks later by applications of a 1% solution of croton oil in benzene once a week in a dose of 0.02 ml until the 20th week of the experiment. In series III BP was applied in a dose of 0.02 ml weekly until the 24th week of the experiment. The following parameters were determined: The time of appearance of the first papilloma and the first carcinoma, the mean latent period of their delopment, the mean number of papillomas per mouse until the time of appearance of the first carcinoma, the percentage of mice with papillomas in the 20th week, and the percentage of mice with carcinomas in the 24th week. Statistical analysis was carried out by Student's method.

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