DIURNAL RHYTHMS OF CELL PROLIFERATION IN THE EARLY STAGES OF LIVER CARCINOGENESIS INDUCED IN MICE BY ORTHOAMINOAZOTOLUENE

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The diurnal rhythm of mitotic activity and of the number of DNA-synthesizing cells was studied by an autoradiographic method, using thymidine-^{3}H, in mouse hepatocytes in the early stages of carcinogenesis of the liver induced by orthoaminoazotoluene. The rhythms of fluctuation of mitotic activity in the control animals in both the first (irregular diffuse hyperplasia) and the second (focal proliferation) stages of carcinogenesis in the liver had a distinct monophasic character with a maximum of the number of mitoses in the early morning and a minimum in the evening or night. Rhythms of DNA-synthesizing cells under normal conditions and in the first stage of carcinogenesis of the liver were monophasic in character with a maximum in the afternoon and evening, respectively. In the second stage of carcinogenesis the rhythm was characterized by the appearance of a second maximum in the early morning. The mean diurnal values of the two indices increased in the second stage of carcinogenesis.

KEY WORDS: orthoaminoazotoluene; liver carcinogenesis; diurnal rhythm of mitosis and of DNA-synthesizing cells.

The characteristics of the dynamics of DNA synthesis and of cell division and also the kinetics of target cell populations play important roles in carcinogenesis [5, 7, 10].

The character of the diurnal rhythm of mitosis and of changes in the number of DNA-synthesizing cells in normal tissues has been studied in detail [1, 3, 11]. Evidence continues to be found that tumors also have a diurnal rhythm of cell division [2, 8] and show changes in the number of DNA-synthesizing cells [4, 9]. However, this process has hardly been investigated at all so far as the successive stages of neoplastic transformation of target cells during chemical carcinogenesis are concerned.

The diurnal dynamics of the number of DNA-synthesizing cells and of the number of dividing hepatocytes in the early stages of carcinogenesis in the liver was studied.

EXPERIMENTAL METHOD

Noninbred male mice with a mean weight of 20-25 g were used. Orthoaminoazotoluene (OAAT) was injected through the mouth directly into the esophagus from a syringe with a curved blunt needle, as a 1% oily solution in a dose of 0.1 ml to each animal three times a week. The mice of group 1 received OAAT for 1 month (total dose 12 mg per animal), those of group 2 for 5 months (total dose 60 mg per mouse); the third group formed the control. The mice received a natural diet and were kept under conditions of illumination. The animals were killed on the 3rd day after the end of OAAT administration. Thymidine-^{3}H (USSR) with a specific activity of 19.8 Ci/mmole was given in a dose of 0.5 μCi/g body weight 1 h before sacrifice. At each time of the investigation seven or eight mice were killed. Pieces of the liver were fixed in Carnoy's solution. Sections 5 μ thick were coated with type M emulsion. The exposure was 50 days. The autoradiographs were stained with Carazzi's hematoxylin. Cells with more than 3 grains of silver above their nucleus were regarded as labeled. The mitotic index (MI) and the index of labeled nuclei (ILN) were determined in promille, by counting 10,000-20,000 cells in each animal. The experiments were carried out in January and February.

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TABLE 1. Diurnal Changes in MI and ILN of Hepatocytes of Control Mice and Mice in Early Stages of Liver Carcinogenesis Induced by Administration of OAAT for 1 and 5 months (M ± m)

Time of days	Control mice				Administration of OAAT for 1 month				Administration of OAAT for 5 months			
	MI	P	ILN	P	MI	P	ILN	P	MI	P	ILN	P
10 a.m.	0,80±0,17		4,40±0,41		0,18±0,01		2,10±0,37		0,65±0,06		5,35±1,63	
				0,060						0,141		0,389
1 p.m.	0,60±0,17		3,30±0,51		0,18±0,01		1,80±0,07		0,47±0,15		3,80±1,10	
										0,387		0,220
4 p.m.	0,46±0,11		4,10±0,77		0,19±0,01		2,41±0,77		0,64±0,25		6,79±1,90	
				0,010				0,141		0,253		0,187
7 p.m.	0,31±0,01		2,20±0,26		0,14±0,04		4,35±0,90		0,34±0,04		3,74±1,10	
		0,015						0,009		0,002		0,040
10 p.m.	0,17±0,04		2,30±0,52		0,13±0,01		1,23±0,31		0,14±0,03		1,77±0,47	
				0,560						0,099		0,001
1a.m.	0,21±0,07		1,90±0,52		0,10±0,02		1,04±0,28		0,61±0,34		1,91±0,31	
		0,058		0,497		0,001						0,001
4 a.m.	0,39±0,09		2,40±0,34		0,43±0,03		0,82±0,14		0,73±0,12		8,51±1,93	
		0,117		0,187						0,218		0,293
7 a.m.	0,32±0,12		1,76±0,22		0,34±0,07		1,07±0,18		0,47±0,14		6,14±1,81	
Mean diurnal values	0,40±0,07		2,75±0,34		0,21±0,01		1,85±0,48		0,51±0,05		4,80±0,81	

$P_{10p.m.-4a.m.} = 0.028$ $P_{10a.m.-7a.m.} = 0.001$ $P_{4a.m.-10a.m.} = 0.002$ $P_{10p.m.-4a.m.} = 0.01$ $P_{4a.m.-1p.m.} = 0.017$
$P_{10a.m.-10p.m.} = 0.001$ $P_{10a.m.-7p.m.} = 0.001$ $P_{1p.m.-7p.m.} = 0.026$ $P_{4p.m.-10p.m.} = 0.07$ $P_{4p.m.-10p.m.} = 0.004$

EXPERIMENTAL RESULTS

One month after the beginning of OAAT injection, the normal pattern of the liver structure was lost because of proliferation of "oval cells"; marked polymorphism of the liver cells was observed. From time to time greatly enlarged liver cells with large hyperchromic nuclei were seen. Hyperplasia of the Kupffer cells and proliferation of the epithelium of the bile ducts also were characteristic. The presence of regenerative changes along with degenerative processes in the early stages of carcinogenesis led Shabad [6] to describe this stage as the stage of diffuse irregular hyperplasia.

Against the background of the picture described above, multiple foci consisting of homogeneous and usually basophilic cells, rather smaller in size than usually, with round nuclei containing one or two nucleoli, appeared in the parenchyma of the liver of the mice receiving OAAT for 5 months. A gradual transition was observed from these nodules to the surrounding liver tissue. The second stage of carcinogenesis, the stage of focal proliferation [6], is connected with the development of nodular hyperplasia.

The results of a study of cellular proliferation are given in Table 1. They show that the diurnal rhythm of of hepatocytes synthesizing DNA in the control animals rose to a single maximum of ILN in the period between 10 a.m. and 4 p.m. and fell to a minimum between 7 p.m. and 7 a.m. The diurnal changes in MI had the same clear monophasic character: a gradual rise of MI starting at 4 a.m. up to a maximum at 10 a.m. and a period of minimal values of MI between 10 p.m. and 1 a.m. A significant increase in the values of MI at 4 a.m. was observed 18 h after the maximal values of ILN.

The diurnal rhythm of ILN of the hepatocytes in mice after administration of OAAT for 1 month also was monophasic in character with a maximum at 7 p.m. and a minimum at 10 p.m. to 7 a.m. There was thus a phase shift of 3 h in the rhythm relative to maximum for ILN of the hepatocytes of normal animals. The character of diurnal changes in MI of the hepatocytes of this group of animals and of the control group of mice was similar. Maximal values of MI in the experimental group were observed at 4-7 a.m. and minimal values between 7 p.m. and 1 a.m. The ILN reached a maximum 9 h before the maximum of MI.

After administration of the carcinogen for 5 months the diurnal rhythm of the DNA-synthesizing cells began to exhibit two maxima of ILN, at 4 p.m. and 4 a.m. Diurnal changes in MI of the hepatocytes in the second stage of development of carcinogenesis were analogous to the changes in MI in the control animals (maximal values between 4 and 10 a.m., minimal between 7 and 10 p.m.). The maximum of ILN was observed 12 h before the maximum of MI.

Comparison of the mean diurnal values of ILN and MI in the experimental and control series showed that following administration of OAAT for 1 month there was a decrease in the mean diurnal values of MI and ILN,

although it was not significant (P = 0.045 and 0.072 respectively). The reason for this could be, as several workers have shown [7, 10], the toxic action of the carcinogen on sensitive target cells. The mean diurnal values of MI and ILN of hepatocytes characteristic of the stage of focal proliferation were more than twice as high (P = 0.002 and 0.001 respectively) as in the earlier stage of carcinogenesis, reflecting the increase in the number of cells in the various phases of the mitotic cycle.

The sequence of changes studied in the process of carcinogenesis in the liver tissue can thus be represented as follows: in the first stage there is a small decrease in the number of cells participating in proliferation, after which their number rises sharply in the second stage of carcinogenesis; in both stages the interval between the maxima of ILN and MI is reduced, in all probability because of an increase in the rate of the proliferation of the hepatocytes. By contrast with the monophasic rhythm of the DNA-synthesizing hepatocytes under normal conditions and in the first stage of carcinogenesis, in the stage of focal proliferation an additional maximum of ILN appears in the early morning. This may be due to the appearance of a new subpopulation of hepatocytes during the formation of foci of nodular hyperplasia [7]. The existence of a diurnal rhythm of DNA synthesis and of the number of dividing cells is evidence of partial preservation of the sensitivity of the liver cells in the early stages of carcinogenesis to the action of factors controlling the intensity of cell proliferation and causing cells to enter the various phases of the mitotic cycle unequally at certain times of day.

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