EFFECT OF AZATHIOPRENE ON DNA SYNTHESIS, MITOTIC ACTIVITY, AND HYPERTROPHY OF CELLS IN THE REGENERATING LIVER AND INTACT SPLEEN OF RATS

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Two-thirds of the liver was removed from August rats, and a suspension of azathioprene in 1% carboxymethylcellulose was injected in a dose of 40 mg/kg. The level of DNA synthesis was determined after 22, 48, 72 h with the aid of thymidine-H^3, and the mitotic activity and the dimensions of the cells and their nuclei were determined in the regenerating liver and intact spleen. Injection of azathioprene led to a decrease in the number of DNA-synthesizing cells in the regenerating liver and spleen and depressed mitotic activity of the hepatocytes. Considerable development of hypertrophy of the cells and nuclei under the influence of azathioprene also were observed in the hepatocytes of the regenerating liver and the small lymphocytes of the white pulp of the spleen, i.e., the weight of the regenerating liver of the rats receiving azathioprene was restored by hypertrophy as well as mitotic division of the cells.

KEY WORDS: azathioprene; hypertrophy of hepatocytes; regeneration of the liver; inhibition of proliferation.

Investigations have recently been published in which the inhibitory action of azathioprene, a known immunodepressant, on DNA synthesis and proliferative activity in regenerating tissues and on DNA snythesis in the intact kidney, spleen, thymus, and lymph glands has been demonstrated. This compound gave rise to marked hypertrophy of the cells [1-3].

Since no sufficiently precise quantitative assessment of cellular hypertrophy has been undertaken during the study of the action of azathioprene on the various organs and tissues, an investigation was carried out in order to study the effect of azathioprene on DNA synthesis, proliferative activity, and cellular hypertrophy of the regenerating liver and intact spleen.

EXPERIMENTAL METHOD

Male August rats weighing 191 ± 29 g were used. Two-thirds of the liver was removed from all the animals, which were then divided into two groups – control and experimental. The experimental animals received a suspension of azathioprene in 1% carboxymethylcellulose (40 μg/kg). Three injections of azathioprene were given: 12 h and 2 h before the operation, performed at 10 a. m., and 2 h before sacrifice of the animals. The control rats received only 1% carboxymethylcellulose at the same time. The suspension of azathioprene in 1% carboxymethylcellulose was given to the animals by gastric tube. Control and experimental rats were killed in groups of 4-6 at a time 22, 48, and 72 h after partial hepatectomy. Thymidine-H^3 in Hanks's solution was injected intraperitoneally in a dose of 1 μCi/g into the rats 1 h before sacrifice (at 10 p. m.). Changes in the body weight and in the absolute and relative weights of the regenerating liver and spleen were studied during the experiments. The index of labeled nuclei (ILN) was determined by counting the labeled hepatocytes in 7000 cells of the regenerating liver and the labeled cells

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TABLE 1. Effect of Azathioprene on Weight of Regenerating Liver and Intact Spleen of Rats (M±m)

Time after resection of liver (h)	Group of animals	Wt. of rat (in g)	Wt. of regenerating liver		Wt. of spleen	
			absolute (in g)	relative (in %)	absolute (in g)	relative (in %)
22	Control	175±1,1	4,0±0,30	2,3±0,16	475±21,75	0,28±0,08
	Exptl.	174±3,4	3,5±0,01	2,0±0,12	400±29,60	0,23±0,11
48	Control	178±1,8	5,5±0,35	3,1±0,42	675±38,00	0,38±0,30
	Exptl.	163±6,0	4,7±0,30	2,9±0,02	500±57,05	0,31±0,04
72	Control	226±1,0	7,4±0,21	3,3±0,08	—	—
	Exptl.	170±7,6	5,8±0,70	3,4±0,11	725±61,50	0,43±0,02

TABLE 2. Effect of Azathioprene on MI and Dimensions of Cells and Nuclei of Regenerating Liver and Intact Spleen of Rats (M±m)

Time after resection of liver (h)	Group of animals	MI of regenerating liver (in %)	Area of hepatocyte in μ^2		Area of small lymphocyte (in μ^2)	
			cell	nucleus	cell	nucleus
22	Control	1,70±0,54	286±2,9	50±4,3	20±0,1	6±0,1
	Exptl.	0,73±0,16	302±9,1	56±2,7	17±0,1	11±0,1
48	Control	22,55±3,57	207±4,2	37±1,8	27±0,2	9±0,1
	Exptl.	9,70±1,48	486±15,4	70±8,2	36±0,1	11±0,7
72	Control	8,86±0,83	228±8,1	37±1,3	27±0,2	9±0,1
	Exptl.	15,40±0,93	269±14,6	52±2,3	36±0,1	11±0,5

among 3000 cells in the white pulp of the spleen. The number of DNA synthesizing hepatocytes was counted among 6000 cells of the regenerating liver and 3000 cells in the white pulp of the spleen. The mitotic index (MI) was expressed per 1000 cells. Two diameters of the hepatocytes and their nuclei in the regenerating liver and of the small lymphocytes in the white pulp of the spleen were measured with an ocular micrometer and the area of the cells and their nuclei were calculated in square microns. The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

No significant differences in body weight or absolute and relative weight of the regenerating liver and intact spleen were found in the experimental and control animals at any time of the investigation (Table 1). Consequently, the rate of restoration of the mass of the regenerating liver in the animals receiving azathioprene was the same as in the rats receiving 1% carboxymethylcellulose. Azathioprene likewise caused no significant change in the weight of the spleen.

In the rats receiving azathioprene and in the control animals the labeled cells were concentrated chiefly at the periphery of the hepatic lobule. Under the influence of azathioprene the number of DNA-synthesizing hepatocytes fell significantly (by 70%). In the control series 22 h after resection ILN was 30%, compared with only 8% in the experimental series ($P=0.006$). In the white pulp of the spleen azathioprene inhibited DNA synthesis by more than half: in the control animals ILN was 7.3% and in the experimental animals 1.9% ($P=0.003$).

The study of mitotic activity in the regenerating liver of the experimental and control animals showed that 22 h after resection, MI was not significantly different from the control: 1.70% in the control and 0.73% in the experiment ($P=0.073$). In the control rats 48 h after the operation, MI was increased and reached its maximum of 22.5% ($P=0.000$), after which it fell to 8.9% at 72 h ($P=0.005$). Meanwhile in the experimental animals receiving azathioprene, MI rose gradually throughout the period of investigation to reach its maximum (15.4%) 72 h after resection of the liver ($P=0.000$; Table 2).

The mean area of the hepatocytes and their nuclei 48 h after resection was reduced in the regenerating liver of the control animals ($P=0.000$), for at this period the more intensive formation of new, young cells, smaller in size than the undivided hepatocytes, could be observed. The size of the cells and their nuclei 72 h after the operation was increased, evidently in connection with the beginning of polyploidization of the regenerating liver cells.

Injection of azathioprene into the animals gave rise to considerable hypertrophy of the cells of the regenerating liver and intact spleen. Some decrease in hypertrophy of the hepatocytes ($P=0.000$) and their

nuclei ($P=0.002$) in the experimental rats 72 h after the operation compared with 48 h can be explained by the appearance of many young cells, formed as a result of mitosis, the small size of which was reflected in the results of determination of the mean values of cell hypertrophy; however, the dimensions of the cells and nuclei in the experimental series still exceeded the control values ($P=0.037$).

The results of this investigation showed that administration of azathioprene to rats had no significant effect on the weight of the regenerating liver and intact spleen but depressed the rate of DNA synthesis in those organs. Meanwhile, under the influence of azathioprene, mitotic activity was retarded in the regenerating liver and cellular hypertrophy distinctly developed.

The causes of hypertrophy of the cells and nuclei after administration of azathioprene have not yet been explained, and special investigations must be carried out to determine them. Since under the experimental conditions used DNA synthesis was inhibited in the liver, the hypertrophy of the hepatocytes was evidently not connected with their polyploidization.

Under the influence of azathioprene regeneration of the liver in rats in the early stages after resection thus takes place on account of the marked hypertrophy of the hepatocytes rather than by stimulation of their mitotic activity.

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