RELATIONSHIP BETWEEN CELLULAR AND INTRACELLULAR FORMS OF REPARATIVE REGENERATION IN THE DEGENERATING LIVER

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UDC 616.36-003.8-003.93

Recovery of the liver after degeneration takes place by formation of cells de novo and also by intensification of intracellular regenerative processes.

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Until recently it was generally considered that regeneration of liver tissue takes place mainly through cell multiplication. Now, however, work has been published showing that hypertrophy of the hepatocytes also plays a very important role in compensation of the disturbed liver functions after injury [2, 3, 5, 6, 9]. This process consists essentially of hyperplasia of the intracellular organoids and nuclear apparatus and its special features are polyploidization of the nuclei [1], the appearance of binuclear cells [6], and an increase in the number of cytoplasmic structures [8]. It is accompanied by an increase in the intensity of DNA synthesis in the nuclei, as autoradiographic studies have confirmed [4]. This type of regeneration is described as intracellular [7].

There is reason to suppose that intracellular regeneration plays a particularly important role in repair processes in liver tissue after diffuse degeneration, i.e., in a type of liver lesion which is frequently encountered in clinical practice.

It was considered that the importance of each of these types of regenerative reaction in liver tissue after degeneration could be examined by a combined study of the level of mitotic activity of liver cells and the intensity of DNA synthesis in them. If an increase in the number of mitoses in the regenerating liver tissue were accompanied by a parallel increase in the intensity of DNA synthesis, it could be concluded that regeneration took place principally through cell multiplication, and that the increase in DNA synthesis was to provide for this process. If, however, it was discovered that the number of labeled liver cells was substantially higher than the number of mitotically dividing cells, this would be precise evidence that intracellular hyperplastic processes played an important role in restoring the normal functions of the liver cells.

The object of the present investigation was thus to study the relationships between cellular and intracellular forms of regeneration in the liver by comparing the autoradiographic findings with the results of analysis of the mitotic activity of the cells.

EXPERIMENTAL METHOD

Experiments to study the mitotic behavior of the liver were carried out on 27 sexually mature male mice weighing 20-22 g, of which 8 were control and 19 experimental animals. The latter received ten subcutaneous injections of 0.2 ml of a 40% solution of CCl_4 on alternate days. The mice were sacrificed by decapitation on the 3rd day after the final injection of CCl_4.

At 8 a.m., or 1 h 15 min before sacrifice, the mice were given an intraperitoneal injection of thymidine-H^3 in a dose of 1.5 μCi/g body weight (specific activity 9 μCi/ml). One ampule of the preparation contained 1 μCi thymidine-H^3 in 1 ml physiological saline. The total dose of precursor for each animal was 30-33 μCi.

A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Active Member of the Academy of Medical Sciences of the USSR A. A. Vishnevskii.) Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 3, pp. 103-105, March, 1969. Original article submitted June 26, 1968.

TABLE 1. Changes in Mitotic Index (MI) (in %) In Hepatocytes of Mice After Ten Injections, each of 0.2 ml 40% CCl_4, on Alternate Days

Group of mice		Time of day (a.m.)							
		0	2	4	6	7	8	9	10
Experimental	$M \pm m$ n	2,4±1,2 (9)	2,6±1,2 (6)	1,02±0,17 (9)	4,11±0,27 (9)	0,26±0,06 (6)	1,36±0,25 (6)	0,66±0,25 (3)	0,57±0,08 (9)
Control	$M \pm m$ n	2,8±1,86 (3)	2,06±0,16 (3)	0,2±0 (8)	6,3±1,48 (3)	0,46±0,07 (3)	0,26±0,07 (3)	0,95±0,05 (4)	0,06±0,04 (3)
P		84%	68%	0,8%	20%	2%	0,3%	28%	0,1%

The animals were sacrificed 1 h 15 min after injection of the isotope. Pieces of liver were fixed in Carnoy's fluid and embedded in paraffin wax. Sections 6-8 μ in thickness were used for preparing autoradiographs. After removal of the paraffin the sections were coated with NIKFI type M fine-grain photosensitive emulsion and exposed in a refrigerator at 4 0 6° for 35-45 days in light proof containers. Development was by the method recommended by NIKFI Motion Picture Research Institute). The autoradiographs were stained with hematoxylin and eosin and then examined in the optical microscope with a magnification of 1000×. Cells were counted as labeled if at least five tracks were found above their nuclei. In each section 3000 cells were counted.

Fluctuations in mitotic activity of the liver cells during the 24-h period were studied in mice sacrificed at midnight and at 2, 4, 6, 7, 8, 9, and 10 a.m.. The liver was fixed in Zenker-formol, embedded in paraffin wax, and histological sections were stained with hematoxylin and eosin. To determine the level of mitotic activity the number of mitoses was counted in 5000 hepatocytes in each section.

EXPERIMENTAL RESULTS

The results of counts of the mitoses are given in Table 1.

Comparison of the number of mitoses in the hepatocytes of mice of the experimental and control, intact mice sacrificed at midnight and 2 a.m. revealed no statistically significant difference in the levels of their mitotic activity.

Analysis of sections from the liver of mice sacrificed at 4 a.m. showed that the mitotic index (MI) of the animals of this group was 5 times higher than that for hepatocytes of control, intact mice killed at the same time.

The largest absolute increase in level of mitotic activity in the experimental and control animals during the period of investigation was observed at 6 a.m., in agreement with results obtained by Liozner and co-workers [5]. No statistically significant difference was found between the MI for animals of the experimental groups at this period. At 7 a.m. a sharp decrease in the absolute values of MI was observed for the animals of both experimental and control groups.

The values of MI for hepatocytes of the animals of the experimental group again rose slightly 44 h after the end of CCl_4 administration, to reach 1.36%.

Analysis of the data for mitotic activity of the experimental and control animals given in Table 1 shows that MI for the hepatocytes of the former exceeded MI for the hepatocytes of the latter at 4, 8, and 10 a.m. by 5, 5, and 9 times respectively; at 7 a.m., however, MI for the control animals was twice that for the experimental, and at all other times its value was about equal for both groups.

Statistical analysis of the material was undertaken by the method of indirect differences, the significance of the data being calculated by a constant formula.

Meanwhile, the autoradiographic study of the regenerating mouse liver showed that at 8 a.m. the index of labeled hepatocyte nuclei was 10.7%, i.e., 80 times greater than MI for hepatocytes of the experimental group.

Considering the information in the literature concerning mitotic activity of hepatocytes in the regenerating mouse liver, it may be postulated that the great excess of cells synthesizing DNA intensively over

the number of mitotically dividing cells is due to the demands made by the body on the functions of the regenerating liver, i.e., that increased DNA synthesis is essential for maintaining the work of the pathologically changed organ (liver) and a manifestation of the intracellular type of regeneration.

The overwhelming majority of mitoses was found in the hepatocytes, and only solitary mitoses were present in the Kupffer cells and the epithelium of the bile ducts.

These results thus showed that restoration of normal structure and function of the diffusely degenerated liver takes place by a combination of cellular and intracellular forms of regeneration, i.e., by the formation of new hepatocytes and also by changes in their ultrastructure, there being certain grounds for considering, moreover, that the second mechanism may be more important than the first.

LITERATURE CITED

1. V. Ya. Brodskii, Cell Nutrition, Moscow (1966).
2. M. M. Kalashnikova, Changes in Ultrastructures of Parenchymatous Cells of the Rat Liver After Local X-Ray Irradiation and Mechanical Injury, Candidate Dissertation, Moscow (1965).
3. N. Ya. Kovalenko, Histophysiological Changes in the Regenerating Liver of Rats During Pregnancy and Lactation, Author's abstract of Candidate Dissertation, Moscow (1968).
4. L. D. Krymskii, B. V. Vtyurin, A. K. Badikova, et al., Byull. Éksperim. Biol. i Med., No. 9, 105 (1968).
5. L. D. Liozner, Z. A. Ryabinina, and V. F. Sidorova, Byull. Éksperim. Biol. i Med., No. 5, 96 (1959).
6. Z. A. Ryabinina, Cytologic Analysis of Regeneration of the Mammalian Liver, Author's Abstract of Doctoral Dissertation, Moscow (1968).
7. D. S. Sarkisov, Éksper. Khir., No. 2, 3 (1962).
8. D. S. Sarkisov and B. V. Vtyurin, Electron Microscopy of Destructive and Regenerative Intracellular Processes [in Russian], Moscow (1967).
9. V. F. Sidorova, Uspekhi Sovr. Biol., 57, No. 2, 283 (1964).