EFFECT OF KUPFFER CELL BLOCKADE AT DIFFERENT TIMES AFTER PARTIAL HEPATECTOMY ON HEPATOCYTE REGENERATION

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The system of mononuclear phagocytes of the liver was blocked in male Wistar rats weighing 140-160 g by iron carbonyl (brand R-100 F) with a particle size of 1-1.5 μ. The blockade was carried out 2 h before partial hepatectomy and also 3 and 18 h after the operation. Injection of the iron compound before the operation and in the early prereplicative period of regeneration led to substantial delay of the peak of the index of labeled nuclei and mitotic index of the hepatocytes accompanied by a general reduction in proliferative power of the hepatocytes. Blockade of the system of mononuclear phagocytes in the period of intensive DNA synthesis by hepatocytes of the regenerating liver was less effective. The facts are evidence of the important role of the Kupffer cells in regulation of reparative regeneration of the liver.
KEY WORDS: regeneration of the liver; Kupffer cell; DNA; mitosis.

The Kupffer cells belong to the system of mononuclear phagocytes [7] and their relations with the hepatocytes are complex. This can be seen in processes such as bile formation [1], steroid metabolism [9], and so on.

Few studies of the role of the Kupffer cells in reparative regeneration of the liver have yet been published [3]. To examine this problem in the present investigation the Kupffer cells were selectively blocked by a suspension of iron carbonyl (brand R-100 F) with a particle size of 1-1.5 μ [4]. There are indications that particles of this size are ingested by the macrophages of the liver and do not penetrate into lysosomes of other types of cells in the hepatic mesenchyme (endothelial, etc.) [11]. To determine more precisely the nature of the relationship between the Kupffer cell and hepatocytes during restoration of the mass of the resected liver, iron carbonyl was injected before the operation, in the early prereplicative period, and in the period of most intensive DNA synthesis by the hepatocytes and the intensity of hepatocyte proliferation in each of these situations was compared.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 140-160 g in which two thirds of the liver was resected under urethane anesthesia between 9 and 10 a.m. An injection of 1 ml of a 10% suspension of iron carbonyl (R-100 F) was given into the right femoral vein 2 h before the operation (group 1) or 3 h (group 2) and 18 h (group 3) after the operation. The effectiveness of the blockade of the Kupffer macrophages was verified morphologically. In the control series 1 ml of 0.9% NaCl solution was injected. The rats of groups 2 and 3 were given an intraperitoneal injection of [^{3}H]thymidine in a dose of 2 μCi/g body weight (specific activity 20 Ci/mmole) 1 h before sacrifice. The animals were killed 5-8 at a time 24, 32, 48, and 72 h after partial hepatectomy. The mitotic index (MI, in $^0/_{00}$) was determined by counting 5000 hepatocytes, and the index of labeled nuclei (ILN) was calculated for 3000 hepatocytes. In the latter case, sections stained with hematoxylin – eosin were coated with liquid type M (NIIKhimfoto) photographic emulsion and exposed at 4°C for 10 days. The numerical results were subjected to statistical analysis by means of Student's t test.

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TABLE 1. MI of Hepatocytes (in ‰) in Control and after Blockade of Kupffer Cells of Liver at Different Times after Partial Hepatectomy (M ± m)

Group of rats	Time after operation, h			
	24	32	48	72
Group 1				
experimental	Infrequent mitoses	Infrequent mitoses	14,1±1,1*	3,5±0,5*
control	3,3±0,3	18,3±0,6	5,6±0,3	1,1±0,1
Group 2				
experimental	Infrequent mitoses	Infrequent mitoses	11,7±0,6*	4,9±0,2*
control	2,7±0,2	17,4±0,9	5,1±0,5	1,8±0,2
Group 3				
experimental	4,2±0,3*	8,5±0,6*	6,3±0,5*	2,9±0,2*
control	8,2±0,4	15,1±0,3	4,5±0,4	1,4±0,2

*Here and in Table 2, differences between experiment and control are significant.

TABLE 2. ILN of Hepatocytes in Control and after Blockade of Kupffer Cells of the Liver at Various Times after Partial Hepatectomy (M ± m)

Group of rats	Time after operation, h			
	24	32	48	72
Group 2				
experimental	0,03*	2,2±0,3*	10,8±0,2*	5,7±0,3*
control	17,7±0,5	12,1±1,1	8,4±0,8	2,5±0,4
Group 3				
experimental	16,1±0,6*	9,3±0,3	6,1±0,4*	3,7±0,2*
control	21,7±0,8	11,3±1,1	7,6±0,3	2,1±0,2

EXPERIMENTAL RESULTS

The dynamics of MI of the hepatocytes was the same in the control rats of all three groups (Table 1): After 24 h 2-10 ‰ of mitoses were found; the peak of MI occurred after 32 h; by 48 h MI had fallen by more than half, and by 72 h MI did not exceed 2 ‰.

In the experimental rats of groups 1 and 2 practically no mitoses were found in hepatocytes 24 and 32 h after the operation. The largest number of mitoses was found after 48 h, when in the control MI was significantly reduced. In half of the rats of group 3 the dynamics of MI was closer to the control, although as a rule no clear peak of mitoses could be found in them 32 h after the operation. The marked "trail" of mitoses 72 h after the operation will be apparent, and in the control the values of MI were much lower at these times.

Analysis of the autoradiographs revealed maximal values of ILN in the control rats 24 h after the operation (Table 2); with an increase in the period after the operation ILN thereafter fell steadily.

If the iron preparation was injected into the animals 3 h after the operation, ILN 24 and 32 h later was many times smaller than at the same times in the control rats. The maximal values of ILN were found only after 48 h; 72 h after the operation ILN was considerably lower, although it still remained higher at this time than in the control. It is interesting to note that the peak of mitoses in the rats of this group coincided with maximal values of ILN, and as a first approximation this can be regarded as a definite tendency toward autosynchronization of the regenerating hepatocyte pool [5, 6]. If the mononuclear phagocytes were blocked 18 h after the operation, the dynamics of ILN differed only a little from that in the control.

During "loading" of the lysosomes of the Kupffer macrophages a substantial change was thus observed in the course of hepatocyte regeneration. On the general plane, proliferation of the hepatocytes was inhibited, as could be deduced from the delay of the ILN and MI peaks and their lower values than in the control. It is important to emphasize that the effectiveness of blockade of the mononuclear phagocyte system largely depended on its time relative to the periods of hepatocyte proliferation. Blockade clearly inhibited hepatocyte proliferation when this took place immediately before the operation or in the early prereplicative period, i.e., 3 h after the operation. Meanwhile it was less effective in the period of intensive DNA synthesis by the hepatocytes of the regenerating liver.

When the lysosomes of the hepatic macrophages are loaded with foreign material, these cells evidently lose much of their ability to induce hepatocyte proliferation [2]. This may be connected with a decrease in the access of acid hydrolases of the lysosomes, which participate in the "induction" of an adequate microenvironment for the proliferating hepatocytes, into the extracellular medium. The suggestion that acid and neutral pro-

teases can induce cell growth in vitro has recently been confirmed experimentally [8, 10].

On the other hand, during blockade of the lysosomes of the macrophages by foreign particles, these cells may lose their ability to form lipid metabolites (of the fatty acid type), albumin–bilirubin complexes, and other substances which, under normal conditions, stimulate hepatocyte proliferation [1].

Inhibition of hepatocyte proliferation during blockade of the Kupffer macrophages of the liver may perhaps be due to a change in the ability of these macrophages to metabolize corticosteroids [9], to a disturbance of the microcirculation in the hepatic sinusoids, and to other causes.

Although the mechanism of the abnormalities of hepatocyte regeneration during blockade of the Kupffer cells are not yet clear, the results described above are certain evidence of the important role of the Kupffer cells in the regulation of reparative regeneration of the liver.

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EFFECT OF THYROCALCITONIN AND HYPOXIA ON DNA SYNTHESIS IN CONNECTIVE-TISSUE CELLS OF THE REGENERATING SKIN

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The effect of thyrocalcitonin (TCT) on proliferative activity of the connective-tissue cells of regenerating skin was studied by [^{3}H]thymidine autoradiography under conditions of a normal and reduced partial pressure of oxygen. Constant saturation of the body with exogenous TCT leads to an increase in the number of cells entering the S period of the mitotic cycle, intensification of DNA synthesis, and considerable dilution of the label during the 24-h period of observation. This may reflect the more rapid passage of the cells through individual stages of the mitotic cycle under conditions of a normal partial pressure of oxygen and hypoxia.

KEY WORDS: regeneration of the skin; thyrocalcitonin; hypoxia, proliferation.

Recent investigations have conclusively shown the activating effect of the thyroid hormone thyrocalcitonin (TCT) on regeneration of bone tissue [4, 5] and skin [1]. This effect can be explained by selective action on the fibroblast population [2, 8].

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