COMPENSATION AND RESTORATION IN THE LIVER AFTER SHOCK-INDUCING INJURIES AND STIMULATION OF MONONUCLEAR PHAGOCYTES

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UDC 616.36-02:613.863]-07:616.36-003.93+616.155.3-008.13

KEY WORDS: shock; liver; intercellular interactions; compensatory and restorative processes; stimulation of mononuclear phagocytes

Despite the abundant factual evidence of marked disturbances of liver structure and function in extremal states, the character and intensity of compensatory and restorative reactions of the organ in the period after shock have received little study. The only communications on the adaptive trend of certain changes in metabolism and ultrastructure of the liver in shock [1, 8] do not reveal the morphological principles governing the restorative period or the role of compensatory reactions in the outcome of post-aggressive states. Correlation between and interdependence of morphological and functional changes in the hepatocytes and stellate reticuloendotheliocytes (Kupffer cells), whose cooperative interaction under normal and certain pathological conditions is not disputed, still remain, however, a matter for discussion [4, 12].

This paper describes an attempt to identify structural features and quantitative ratios of liver cells which could reflect the state of compensatory and reparative reactions in the liver in etiologically different types of shock. Within the limits of this task, we were also interested to study the effect of stimulation of the mononuclear phagocyte system (MPS) on regeneration of hepatocytes after shock-inducing injuries.

EXPERIMENTAL METHOD

Cannon's experimental model of traumatic shock and a model of burn shock created by applying metal, heated to 500°C to the depilated dorsal surface, and leading to a burn of the IIIb-IV degree affecting an area of about 20% of the body surface, were used in experiments on noninbred male rats under superficial ether anesthesia. MPS was stimulated by the bacterial polysaccharide prodigiosan, a 0.005% solution of which was injected intraperitoneally 24 h before creation of the experimental model of shock, in a dose of 25 μg/100 g body weight. Morphometric analysis of the liver cells was carried out on preparations stained with hematoxylin and eosin and by Feulgen's method, and also on semithin sections stained with methylene blue–azure II–basic fuchsine. The DNA content in the hepatocyte nuclei was measured on a modified scanning integrating microspectrophotometer [1], taking as the standard of a diploid set of chromosomes the results of mathematical analysis of microspectrophotometric data for lymphocytes in the same liver preparations. For autoradiographic investigation, ^{3}H-thymidine with specific radioactivity of 1790 TBq/mole was injected intraperitoneally into the experimental animals 1 h before sacrifice, in a dose of 1 μCi/g body weight, and the liver sections were then coated with type M photographic emulsion and exposed for 3-4 weeks at 4°C. The ultrastructural features of the tissue were studied in objects fixed in 2.5% glutaraldehyde and 1% osmic acid, and embedded in Epon 812. Ultrathin sections stained with uranyl acetate and lead citrate were studied in the IEM-100B electron microscope.

Kazan' State Medical Institute, Ministry of Health of the Russian Federation. (Presented by Academician of the Russian Academy of Medical Sciences D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 8, pp. 136-138, August, 1992. Original article submitted December 20, 1991.

 0007-4888/92/0008-1092$12.50

TABLE 1. Relative Percentages of Binuclear Hepatocytes, Stellate Reticuloendotheliocytes, and DNA-Synthesizing Cells in Rat Liver after Shock-Inducing Injuries

Type of cell	Control	Traumatic shock, h				Burn shock, h			
		12	24	48	72	12	24	48	72
Binuclear hepatocyte	11,3±2,4	11,9±2,2	14,9±1,9	19,1±0,8*	16,0±1,4*	12,4±2,8	13,9±1,8	15,4±1,4	21,2±2,6*
Stellate reticuloendotheliocyte	8,9±1,6	9,4±1,8	14,3±0,6*	17,9±1,1*	14,2±0,3*	9,3±1,2	10,4±1,2	13,7±1,3*	17,9±1,0*
Index of labeled nuclei	0.18±0.02	0,24±0,04	0,88±0,1*	1,8±0,4*	0,61±0,1*	0,22±0,03	0,3±0,07	0,92±0,1*	1,6±0,3*

Legend. Number of rats at each time of experiment 9-10. *$p < 0.05$ compared with control.

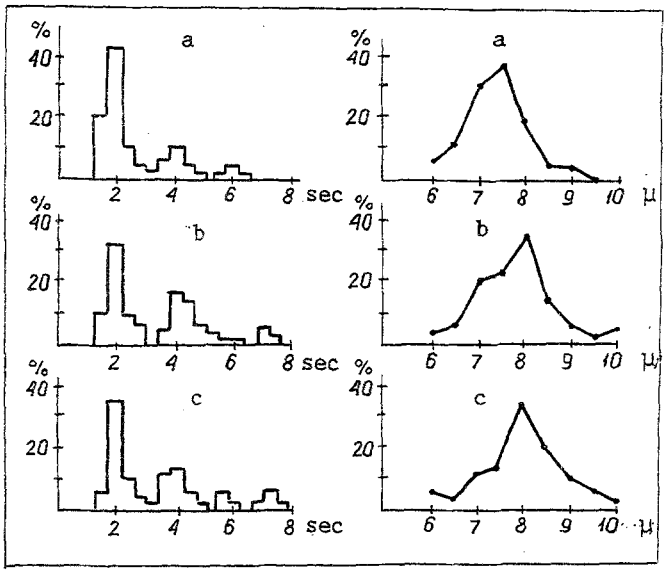

Fig. 1. Histograms of DNA content in hepatocyte nuclei ana variance curves of nuclear size in traumatic (48 h) and burn (72 h) shock. Abscissa, values of ploidy classes and nuclear size classes; ordinate, relative percentages of nuclei of each class; a) control, b) traumatic shock, c) burn shock.

EXPERIMENTAL RESULTS

As the data in Table 1 show, by the end of the 1st day after mechanical trauma the index of labeled nuclei in the liver increased, and later there was an increase in the number of binuclear liver cells. A similar but slower process could be observed after burn trauma, corresponding to the more prolonged course of the torpid phase of burn shock in rats. The results of microspectrophotometry and karyometry demonstrated an increase in the content of Feulgen-positive DNA in the hepatocyte nuclei, an increase in ploidy of the liver cells, and a shift of the variance curves of nuclear size to the right (Fig. 1). Besides the changes mentioned above, an increase in the number of nucleoli was observed in the hepatocyte nuclei (1.59 ± 0.06 in the experiment, 1.22 ± 0.09 in the control, $p < 0.05$), and the mitotic index showed a very small rise to 0.2-0.37‰. At these stages of the postshock period, electron-microscopic investigation of the dark hepatocytes revealed the well-known combination of ultrastructural bases of activation of compensatory and adaptive reactions coupled with regenerative hyperplasia of the intracellular organelles [7]. It is important to note that these cytoarchitectonic and ultrastructural features could be detected only in the liver of survivors, i.e., of animals relatively resistant to shock. In the liver of rats which died or were sacrificed in the terminal phase, reactions of injury Predominated over restorative processes.

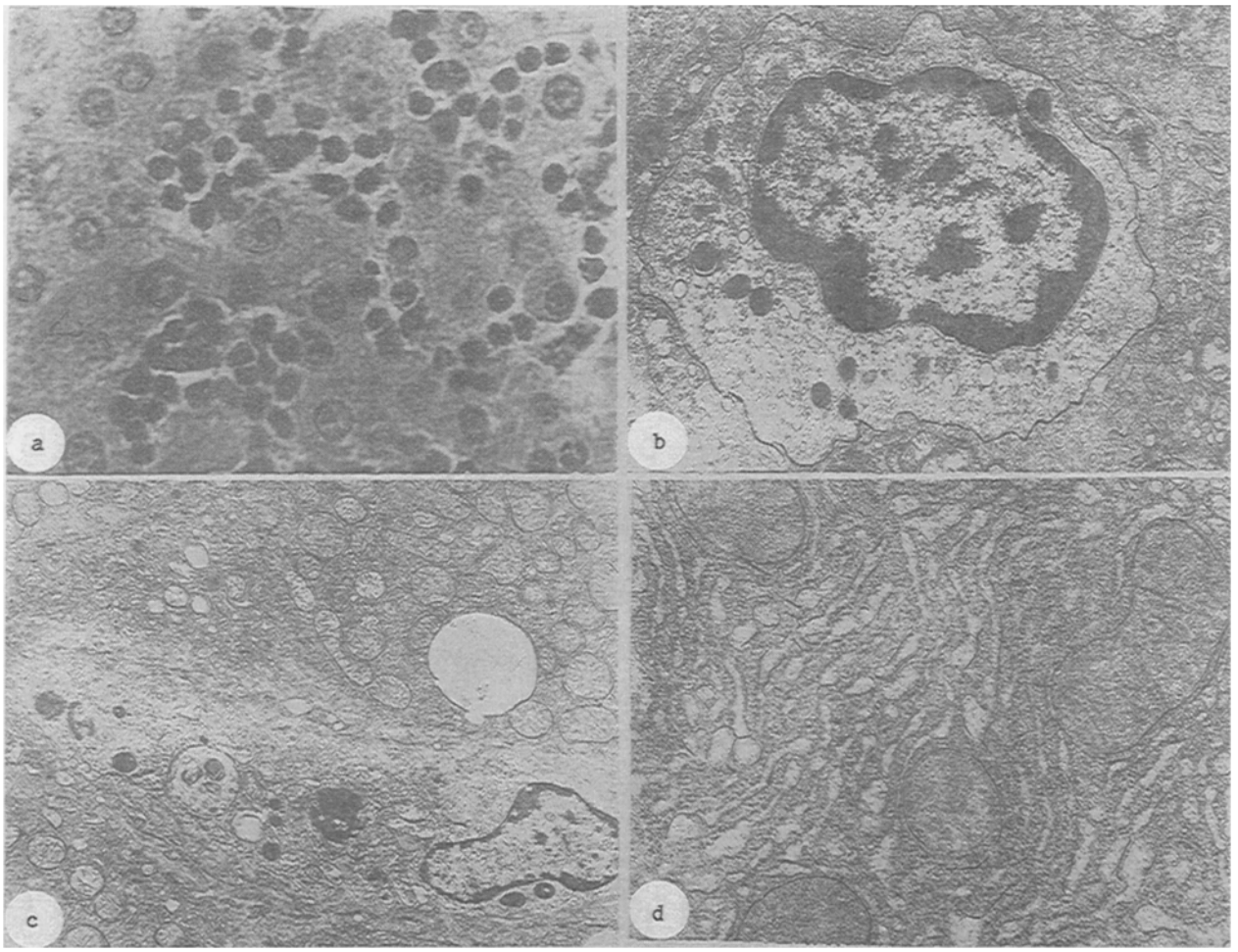

Fig. 2. Changes in liver in post-shock period, after preliminary stimulation of MPS: a) Inflow of mononuclears into liver sinusoids (hematoxylin and eosin, 300×); b) active monocyte with cytoplasmic outgrowths and with accumulation of ribosomes and primary lysosomes (8000×); c) active stellate reticulocytes with multiple cytoplasmic villi and hyperplasia of ribosomes and lysosomes in different stages of transformation, and polymorphism and hyperplasia of mitochondria in adjacent hepatocytes (6700×); d) hepatocyte with marked hyperplasia of compactly arranged rough cytoplasmic reticulum (12,000×).

It is a noteworthy fact that the increase in regenerative potential of the parenchymatous cells correlated positively with the increase in the number of stellate reticuloendotheliocytes (SR) and their precursors, which are components of MPS, in the liver. In both types of injury, recovery of the animals from a state of shock was accompanied by adaptive reorganization of the ultrastructure of SR, evidence of activation of the functional state of these cells, and suggesting that SR have a stimulating effect on hepatocyte regeneration. The results of experiments with preliminary stimulation of MPS confirmed this conclusion. They showed that the development of shock, after preliminary stimulation of MPS, was accompanied by an increased inflow of mononuclears into the liver sinusoids (Fig. 2a); a large proportion of these cells were active monocytes (Fig. 2b). After a period of shock lasting 24 h the number of SR in the liver had risen to 20-22%, and this was accompanied by parallel activation of their intracellular organelles and also hyperplasia and polymorphism of the mitochondria in adjacent hepatocytes (Fig. 2c). The state of the rough cytoplasmic reticulum of the liver cells was very typical, and reflected a strain on synthetic and secretory processes (Fig. 2d). The ultrastructural features of the hepatocytes confirmed the possible intensification of energy metabolism and of the protein-synthesizing function of the liver in response to stimulation of MPS by prodigiosan [6]. Under these conditions we also found acceleration and intensification of the reparative reactions of the liver. The peak of intensification of the regenerative potential of the hepatocytes occurred 24 h after traumatic shock and on the 2nd day after burn shock. During this period there was an increase in the number of mononuclear polyploid cells,

accompanied by a decrease in the relative percentage of binuclear hepatocytes. The results of microspectrophotometry showed that the total number of tetraploid and octaploid cells was 59.6% and 11.2% respectively on average in traumatic shock, and 56.2% and 8.8% in burn shock. The mitotic index in both groups of observations varied between limits of 0.2 and 0.3‰. It is an interesting fact that mobilization of the regenerative powers of the liver in the post-shock period, after preliminary stimulation of MPS, was accompanied by stabilization of the hemodynamic parameters, by a decrease in mortality at the height of the torpid phase of shock by 15-20%, and by an increase in the length of survival of the experimental animals. These findings are in agreement with the increased resistance of the animals with trauma after stimulation of MPS by bacterial polysaccharide observed in [9, 10].

The results of this investigation thus conclusively demonstrated the complex reorganization of the cytoarchitectonics and ultrastructure of the liver in the post-shock period, aimed at compensation of disturbed functions and restoration of the damaged tissue. These processes follow the same, stereotyped course, including proliferation of cells with polyploidization of hepatocytes and hyperplasia of their ultrastructures, as well as activation of the coordinated functions of nucleus and cytoplasm [2, 7]. The rate of development and the intensity of compensatory and repair processes depend largely on reactivity of the individual animal, the severity of the shock, and the specific etiology of the traumatic factor. Close interaction was observed between SR and the parenchymatous cells of the liver. The possibility cannot be ruled out that the triggering mechanism of SR activation in the post-shock period may be the entry of endotoxins, the mechanism of whose stimulating action on MPS has been discussed in sufficient detail in the literature [3, 5], in the blood. Artificial activation of liver macrophages by prodigiosan followed by reproduction of shock confirms this view, by repeating in a more rapid and intensive form the compensatory and adaptive character of morphological changes in the liver. The mechanism of the stimulating effect of SR on hepatocyte regeneration is apparently very complex, but it may be largely mediated through lysosomal enzymes and other biologically active substances of the liver macrophages [4]. Considering the marked depression of the RES of the liver at the height of torpid and burn shock [11, 13-15], the use of the results of this investigation for the continued search for methods and materials aimed at preventing and correcting disturbances of liver morphology and function in the post-shock period would seem to be justified.

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