DESTRUCTIVE AND REPARATIVE PROCESSES IN THE LIVER IN ACUTE EXPERIMENTAL PERITONITIS

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Mortality from acute peritonitis (AP) is 10-66.9% [5, 9]. One of the immediate causes of the patients' death is hepatic failure, which develops as a result of the action of toxic substances on the parenchyma of organs [3, 6].

The aim of this investigation was to identify the character of relations between damage and repair of the liver tissue at different stages of AP.

EXPERIMENTAL METHOD

Experiments were carried out on 145 albino rats of which 135 belonged to the experimental and 10 to the control group. Acute fecal peritonitis was produced by the method in [10] (physiological saline was injected intraperitoneally into the control animals). The effect of the strength of the toxic action on pathomorphology of the liver was studied in some of the animals, into which a fecal suspension was injected in higher concentration (2-2.5 times), or repeatedly. The longest duration of the experiment was 10 days. The character of destruction and repair of the liver under conditions of altered immunologic homeostasis was studied in 18 rats, in which peritonitis was accompanied by administration of an immunodepressant (azathioprine), whereas in 15 rats AP was accompanied by administration of an immunostimulator (levamisole). The animals were killed by decapitation under ether anesthesia, but some of them died on the 3rd or 4th day with marked toxic manifestations. Sections through the organs were stained by general histological methods, and histochemical reactions also were carried out for total nucleoproteins (Einarson), fat (Sudan), and acid a-naphthyl acetate esterase according to [15] in the modification [14].

Electron-microscopic and histoautoradiographic investigations were carried out, paying attention to recent recommendations [4, 13]. The morphometric analysis included quantitative counting of the liver cell populations: hepatocytes (HC), stellate reticuloendotheliocytes (SR), lymphocytes (L), and other mononuclears, calculated per 1000 cells. The area of the necrotic foci was measured, using G. G. Avtandilov's planimetric grid and an MOV-1 ocular micrometer. The numerical results were analyzed on the MK-59 computer, with determination of the mean values and their errors ($M \pm m$), expressed in promille.

EXPERIMENTAL RESULTS

Acute experimental peritonitis (AEP) evolved in the animals from serous inflammation of the peritoneum (after 10-16 h) to serofibrinous (on the 2nd-3rd day) and, later, to lesions of a fibrinopurulent or pyonecrotic character. The last was most characteristic of cases with a high dose of toxic exposure, and with suppressed immunologic reactivity. On the 8th-10th day the inflammatory exudate in the peritoneal cavity ceased, with the formation of encapsulated abscesses.

On the 1st day of AEP (reactive stage), besides vascular and marked degenerative changes in the liver, disseminated necrosis of hepatocytes (DNH) developed: for every 1000 normal cells there were 8.51 ± 0.59 necrotic cells (Fig. 1a); the corresponding figure in the control was 0.22 ± 0.004. Significant redistribution of the different classes of liver cells was observed, with some increase in the number of binuclear HC (70.33 ± 1.48), the initial value being 20.72 ± 0.48‰, and in the number or cells with hypertrophied (polyploid) nuclei (27.84 ± 0.6 compared with 16 ± 0.52‰ in the control), and there was marked inhibition of mitotic and synthetic activity of the liver cells in the early stages of AP. The number of L in the lumen of the sinu-

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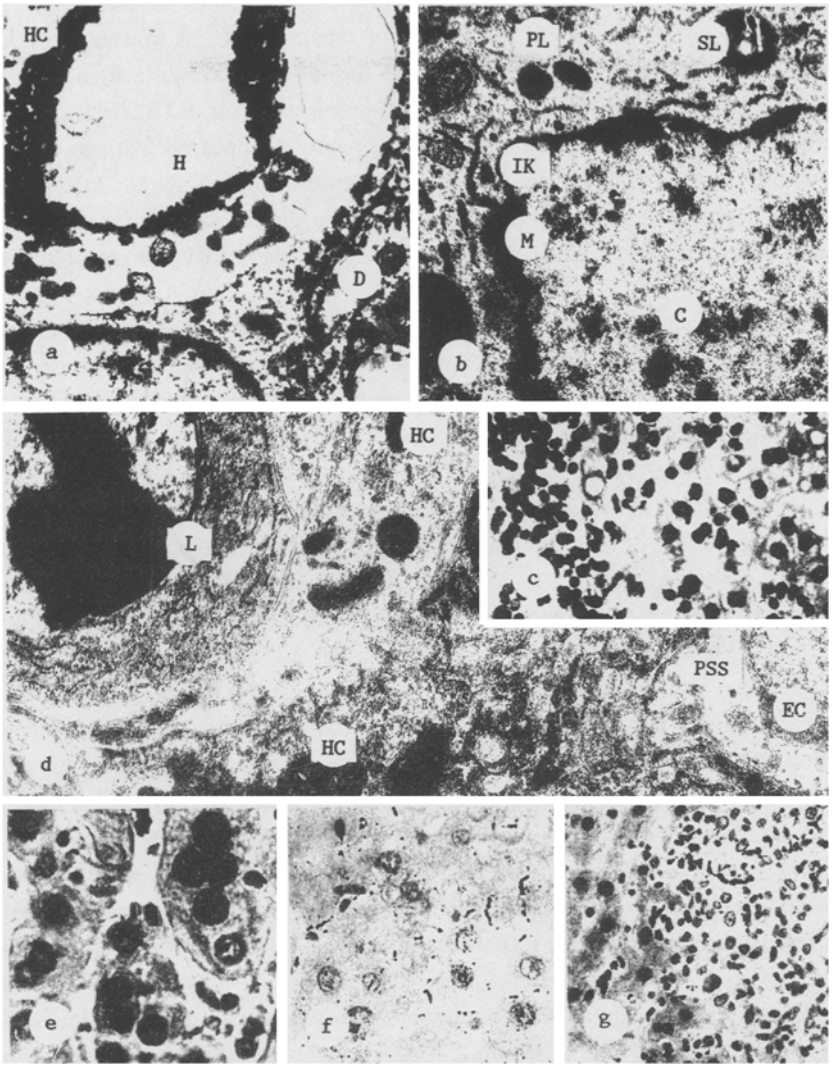

Fig. 1. Morphological characteristics of destructive and reparative processes in the liver in AEP. a) Fragment of necrotic hepatocyte (HC): hydration (H) and destruction (D) of organelles. Reactive stage of AEP. Magnification 18,000; b) fragment of activated SR: condensation (c) and margination (M) of chromatin in nucleus, invagination of karyolemma (IK), and increase in number of primary (PL) and secondary (SL) lysosomes. End of 1st day of AEP. Magnification 18,000; c) sharp increase in number of L inside lobule in AEP accompanied by administration of levamisole. Hematoxylin and eosin. Ocular 3, objective 20; d) migration of L from blood stream between HC: endotheliocyte (EC), widening of perisinusoidal space (PSS). Magnification 12,000; e) accumulation of multinuclear hepatocytes of syncytial types. AEP, accompanied by administration of levamisole. Stained with Einarson's gallocyanin; f) incorporation of ^{3}H-thymidine into hepatocyte nuclei. Second day of AEP. Ocular 3, objective 40; g) multiplication of mesenchymal cells at site of former intralobular focus of necrosis. Tenth day of AEP. Hematoxylin and eosin. Ocular 3, objective 20.

soids and in the perisinusoidal spaces (PSS) rose to 87.17 ± 2.35 (compared with 32.63 ± 1.09‰ in the control). The number of SR was 442.17 ± 2.1‰ (compared with 548.29 ± 0.5‰ in the control). In animals which died, mainly as a result of injection of a high concentration of the fecal suspension or its repeated injection into the peritoneal cavity, destructive changes in the organ increased sharply in severity and predominated over repair processes: the number of mitoses, the labeling index, and the number of binuclear cells were significantly depressed. No increase was observed in the number of L. The SR were swollen and round, their processes were shortened, and their cytoplasm contained an increased number of secondary lysosomes (Fig. 1b).

In the experiments with azathioprine (at the same stages of AEP) the index of destruction was close to the level corresponding to the usual course of AP. However, all the indices of repair of HC, as well as the number of L in the liver (49.53 ± 4.06‰), were depressed. Conversely, in the experiments with levamisole there was a significant increase in the number of L (125.62 ± 0.95‰, Fig. 1b, c) and a significant increase in the parameters of repair of HC (Fig. 1e). After 2 days the destructive changes in the liver were increased and the initial signs of inflammation appeared, with an increase in the number of polymorphs and lymphocytes within the lobules and in the portal tracts. Besides the well marked DNH, multiple intralobular foci of necrosis weze recorded in the liver, having developed on the basis of a disseminated intravascular clotting syndrome and thrombosis of small vessels and sinusoids. The area of necrotic liver tissue, calculated per cm of surface of the section, was 4.25 ± 0.34‰.

Plasma proteins, L, polymorphs, and fragments of their nuclei could be seen in the sinusoids and PSS. The number of SR (442.17 ± 2.1‰) and of ^{3}H-thymidine-labeled nuclei of HC (48.34 ± 0.72‰) was significantly increased (Fig. 1f). The concentration of DNA, total nucleoproteins, and glycogen was reduced in the center of the lobules, whereas in the peripheral zones it remained relatively high. The number of HC with polyploid nuclei reached 39.86 ± 1.06‰, the number of binuclear cells 90.8 ± 0.4‰, and the number of mitoses 2.34 ± 0.015‰.

At the end of the 3rd day the mitotic activity of the liver cells and the labeling index of HC were 3.695 ± 0.03 and 66.8 ± 2.56‰ respectively. After 4.5 days the values of these parameters reached their maximum: 6.48 ± 0.04 and 106.2 ± 3.65‰. The number of L in the liver was 22 times greater than in the control. In cases of spreading fibrino-purulent and pyonecrotic peritonitis the area of destruction of the hepatic parenchyma usually exceeded 12-15%/cm^2 surface of the section, but in some cases it reached 55-60%, which as a rule is fatal. The parameters of repair and cellular infiltration of the organ were sharply depressed.

The most prominent feature in the polymorphic picture of structural changes in the liver on the 4th day of AP, developing in rats treated with azathioprine, was the presence of multiple foci of destruction and suppuration. The mitotic activity of HC was low (1.84 ± 0.01‰), the number of binuclear cells (22.3 ± 1.2‰), of cells with polyploid nuclei (12.98 ± 0.3‰), and of mononuclears in the liver were significantly lower than during the usual course of AP at the same times of the experiment. In the experiment with levamisole a high content of intralobular L (55.7 ± 0.03‰) and SR (543.6 ± 2.4‰) was recorded. The number of binuclear HC (183.3 ± 0.36‰), of cells with polyploid nuclei (55.7 ± 0.37‰), and the number of mitoses (8.96 ± 0.02‰) were significantly increased. Reparative reactions were clearest of all at sites of concentration of L. Destructive processes were ill-defined.

On the 6th-8th days of the experiment limitation of the inflammatory process in the abdomen and its stabilization in the liver were observed. Destructive changes in the liver were reduced. At sites of previous foci of necrosis, multiplication of cells of the fibroblastic series was discovered. Compared with the 4th day of AP the number of binuclear (107.51 ± 2.6‰) and polyploid (30.7 ± 1.1‰) HC was reduced, their mitotic activity was 4.58 ± 0.6‰, and their labeling index 75.8 ± 0.2‰. The number of L in the organ fell to 582.1 ± 2.63 ‰, and the number of SR remained at its previous level (485.6 ± 1.8‰. The experimental animals receiving azathioprine frequently developed hepatitis with abscess formation. In these cases the number of mononuclear cells in the liver fell sharply and the intensity of repair processes was reduced. Conversely, when levamisole was used the regenerative reactions remained at a high level: the number of binuclear and polyploid cells was 130.4 ± 1.3 and 42.3 ± 0.17‰ respectively, mitotic activity was 5.17 ± 0.04‰, and the number of labeled nuclei 101.5 ± 0.31‰. The levels of intralobular L and SR reached 859.7 ± 0.2 and 537.7 ± 1.7 respectively.

By the end of the experiment the inflammatory exudate in the peritoneal cavity as a rule was encapsulated. Destructive changes in the liver were mild and could be reduced simply to focal degeneration and necrosis of single cells. At the site of previous foci of necrosis areas of loose connective tissue could be seen, containing mesenchymal cells (Fig. 1g). The quantitative parameters of lymphoid and leukocytic infiltration and also of reparative regeneration of the liver continued to fall, although they still remained higher than values for animals of the control groups, by a factor of 8.7 and 2.7 times. In the experiments with azathioprine these parameters were lower than initially by 0.92 and 0.35 times, whereas in the experiments with levamisole they were 23.5 and 4.4 times higher.

Thus the intensity of destructive changes in the liver of animals with AP depends directly on the severity of damage to the peritoneum, the strength of toxic action on the parenchyma of the liver, and the degree of depression of immunologic reactivity of the host. In the first 3 days of AEP repair of the liver takes place mainly through intracellular regeneration: the number of binuclear and multinuclear HC and of cells with polyploid nuclei was increased and hyperplasia and hypertrophy of the ultrastructures of the cell was observed. The peak of mitotic activity of HC occurred 4-4.5 days after a single injection of the fecal suspension. Under other experimental conditions (reception of part of the healthy or pathologically changed liver), the

number of mitoses in the liver cells is known to reach a peak during the first 17-24 h [8, 11, 12]. This delay of mitotic division of HC in AEP may be connected with the intensive toxic action of the contents of the pezitoneal cavity on the liver parenchyma, an abrupt disturbance of the hemodynamics and lymphodynamics of the organ [7], and suppression of metabolic and synthetic processes in the liver cells.

In severe forms of inflammation of the peritoneum (characteristically cases with a high concentration or repeated injection of the fecal suspension) and animals with suppressed immunologic reactivity, the area of destruction of the liver amounted to 50-55%, per cm^2 surface area of section, and as a rule this led to death of the animals on the 3rd or 4th day of the experiment. The appearance of massive foci of necrosis under these conditions of AEP was observed to coincide with a fall in the number of L and SR and the appearance of reparative regeneration of the organ, Administration of azathioprine in the experiments led to a decrease in the number of intralobular L, SR, and other mononuclear cells, to aggravation of suppurative and destructive changes in the liver (mainly on the 4th or 5th day), and weakening of regeneration of HC. Conversely, the use of levamisole was accompanied by a significant increase in the number of mononuclears within the lobules, activation of HC proliferation, and earlier restoration of the foci of destruction in the liver. These results confirm the familiar view that L and SR are involved in the regulation of proliferative activity of epithelial cells [1, 2, 8].

LITERATURE CITED

1. A. G. Babaeva, Immunologic Mechanisms of Regulation of Repair Processes [in Russian], Moscow (1972).
2. A. G. Babaeva, Current Problems in Regeneration [in Russian], Ioshkar-Ola (1982), pp. 44-51.
3. N. K. Doronina, V. A. Fishbein, and I. K. Bulanov, Klin. Khir., No. 1, 9 (1979).
4. O. I. Epifanova, V. V. Terskikh, and A. F. Zakharov, Autoradiography [in Russian], Moscow (1977).
5. I. A. Erokhin, Vestn. Khir., No. 7, 3 (1986).
6. L. P. Zhavoronkova, Vestn. Khir., No. 8, 21 (1977).
7. G. S. Ivanova, V. A. Glumova, T. N. Opolonskaya, et al., Intramural Hemodynamics of the Digestive and Excretory Systems [in Russian], Gor'kii (1985), pp. 102-106.
8. B. M. Karlson, Regeneration [Russian translation], Moscow (1986).
9. V. A. Popov, Peritonitis [in Russian], Leningrad (1985).
10. S. S. Remenik, Zdravookhr. Turkmenistana, No. 12, 3 (1965).
11. V. F. Sidorova, Age and the Restorative Capacity of Organs in Mammals [in Russian], Moscow (1976).
12. V. P. Solopaev, Regeneration of the Normal and Pathologically Changed Liver. Experimental Bases of Regeneration Therapy of Liver Diseases [in Russian], Gor'kii (1980).
13. B. Weakley, Electron Microscopy for Beginners [Russian translation], Moscow (1975).
14. M. Chilosi, F. Menestrina, et al., Bas. Appl. Histochem., **25**, 39 (1981).
15. D. M. Knowles and S. Holck, Lab. Invest., **39**, 70 (1978).