EFFECT OF ANTIHEPATOCYTOTOXIC SERUM ON CELLULAR AND INTRACELLULAR REGENERATION OF THE PATHOLOGICALLY CHANGED LIVER

N. V. Il'chevich, I. N. Alekseeva,
T. M. Bryzgina, B. K. Bezprozvannyi,
and T. I. Galenko

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Parameters of regeneration of the liver (mitotic index, DNA synthesis, volume of hepatocyte nuclei, and histological structure) were studied in experiments on adult male rats with liver damage induced by CCl_4 and receiving injections of antihepatocytotoxic serum (γ-AHCS) during regeneration of the liver after CCl_4 poisoning; cellular and intracellular forms were found to predominate at different times. γ-AHCS given in a dose of 0.06 μg protein/100 g body weight per injection stimulates and prolongs regeneration of the liver at both cellular and intracellular levels.

KEY WORDS: liver; antihepatocytotoxic serum; carbon tetrachloride; mitotic index; index of labeled nuclei; volume of nuclei; histological structure.

Regeneration processes in the liver are considered to be the basis for recovery of its disturbed functions [3, 6]. These processes take place at both cellular and intracellular levels (cell proliferation, hypertrophy and hyperplasia of ultrastructures). Both antihepatocytotoxic serum (ACHS) and its γ-globulin fraction (γ-AHCS) have been shown to stimulate processes of regeneration in the liver [1, 2].

The object of the present investigation was to demonstrate the effect of γ-AHCS on the course of regeneration and on relations between its cellular and intracellular forms in liver damage due to CCl_4. The mitotic index (MI) of the hepatocytes, DNA synthesis in parenchymatous and reticuloendothelial cells, the volume of the hepatocyte nuclei, and the histological structure of the liver were determined.

EXPERIMENTAL METHOD

Experiments were carried out on 152 adult female Wistar rats. Three injections of CCl_4 were given, at intervals of 2 days, in a dose of 0.5 ml of a 50% oily solution/100 g body weight, subcutaneously. AHCS was obtained by immunizing rabbits with a saline extract of rat liver parenchyma. The γ-globulin fraction was isolated from the serum with a titer of 1:320-1:400 in the complement fixation text by Kendall's method [5]. γ-AHCS in a dose of 0.06 μg protein/100 g body weight was given to the rats as three injections either on the days after each injection of CCl_4 (series I), or at intervals of 2 days starting with the day after the last injection of CCl_4 (series II).

DNA synthesis was studied by an autoradiographic method based on incorporation of thymidine-^3H. The volume of the hepatocyte nuclei was measured on photographic films taken from histological preparations stained by Feulgen's method. The results of measurement of the diameters of 200-500 nuclei at each time of the experiment were analyzed by computer and histograms obtained. Mean data for the volume of the nuclei for each animal also were calculated. Histological changes were studied in sections stained with hematoxylin-eosin and the number of mitoses in the hepatocytes was counted (in 5000 cells). All investigations were carried out on the same animals; they were killed between 11 a.m. and noon. From three to five rats were killed at each time. The results of the autoradiographic and

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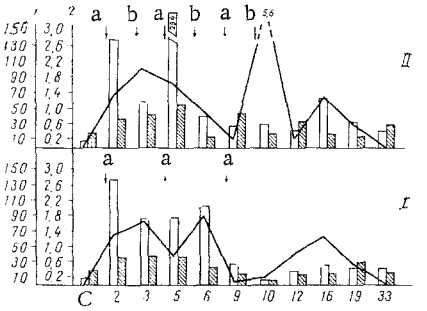

Fig. 1. Changes in MI and ILN in liver of rats with CCl_4 poisoning and receiving γ-AHCS. Columns — ILN: unshaded — parenchymatous cells, shaded — reticuloendothelial cells. Curve shows MI of hepatocytes. I) CCl ; II) CCl_4 + γ-AHCS. a) Injection of CCl_4; b) injection of γ-AHCS. Abscissa, days of experiments; C) control. Ordinate: ILN (in °/₀₀), 2) MI (in °/₀₀).

karyometric investigations were subjected to statistical analysis by means of the Wilcoxon-Mann-Whitney nonparametric criterion [4].

EXPERIMENTAL RESULTS

The first injection of CCl_4 gave rise to severe degenerative and necrotic changes in the peripheral circulatory zones of the hepatic acini. During this same period regenerative processes were observed to develop in the liver. On the day after injection of CCl_4 MI of the hepatocytes was 1.3 (compared with 0 in the control). The index of labeled nuclei (ILN) of the hepatocytes was increased by 19 times. Labeled hepatocytes were found chiefly in the first zones of the acini, outside the limits of necrotic and degenerative changes. In the region of the portal tract and in zones of maximal changes in the parenchyma, moderate proliferation of stellate reticuloendotheliocytes and lymphocytes was observed. ILN of these cells was increased by 2.1 times. Changes in MI and ILN are illustrated in Fig. 1. The response of the nuclei at this time took the form of an increase in the number of nuclei measuring under 100 μ^3 and a decrease in the number of nuclei measuring from 100 to 200 μ^3. The increase in the number of cells with smaller nuclei was evidently the result of more intensive division of the hepatocytes.

The second injection of CCl_4 gave rise to a fresh wave of necrotic changes, but less intensive. ILN and MI of the hepatocytes were much higher than in the control but lower than after the first injection. At this time an increase in size of the nuclei was observed, as shown by the mean data (control 146 μ^3, experiment 212 μ^3; $P = 0.01$) and by the appearance of new size classes of larger nuclei on the histograms.

After the third injection of CCl_4 the necrotic and degenerative changes in the liver were much less marked than after the first two injections. Regenerative changes at the cellular level (MI and ILN) had died away by this time. The reaction of the nuclei, expressed as enlargement, however, was more clearly defined. In the period after the end of administration of CCl_4 ILN and MI gradually returned to normal. A shift toward larger nuclei was observed in the distribution of the nuclei by volume even on the 33rd day of the experiment.

The use of γ-AHCS after injection of CCl_4 (series I) had some effect on the histological picture and on regeneration of the liver. On the day after the first injection of γ-AHCS fatty infiltration of the liver was less marked in both degree and extent. The proliferative reaction of the reticuloendothelium was more intensive. MI and ILN of hepatocytes and reticuloendothelial cells did not differ significantly from their levels at this time in animals receiving CCl_4 only (there was even a tendency for ILN to decrease). The response of the nuclei also was slight. However, in animals receiving γ-AHCS, a second injection of CCl_4

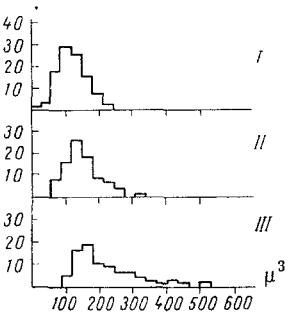

Fig. 2. Histograms of distribution of hepatocyte nuclei by volume in rats receiving γ-AHCS after the end of CCl_4 administration. I) Control; II) CCl_4, 40th day of experiment; III) CCl_4 + γ-AHCS, 40th day of experiment.

gave rise to a much greater increase in ILN and LI of the hepatocytes than in rats receiving CCl_4 alone. Cells synthesizing DNA (both mono- and bi-nuclear) appeared in all zones of the acini, not only in regions unaffected by necrotic and degenerative changes, but also among cells showing fatty and balloon degeneration. At this time some increase in size of the hepatocyte nuclei also was observed.

A second injection of γ-AHCS led to a decrease in ILN and MI of the hepatocytes compared with data at this time for animals receiving CCl_4 alone. However, a marked reaction of the hepatocyte nuclei (enlargement) was observed. New size classes of large nuclei not found in the control or in experiments with administration of CCl_4 alone appeared on the histograms.

On the day after the third injection of γ-AHCS, ILN and MI of the hepatocytes were 6.2 and 28 times higher respectively than for animals not receiving the serum. Changes in the mean volume of the nuclei and their distribution among size classes demonstrated a shift toward smaller nuclei, evidently reflecting intensified cell division. The histological picture of the liver was almost completely restored to normal. By contrast with the effect of CCl_4 alone, after combined administration of CCl_4 and γ-AHCS large cells with less dense cytoplasm were more numerous. On the 16th and 19th days of the experiment ILN of the parenchymatous cells was statistically significantly higher than for animals receiving CCl_4 alone. No significant differences were found in the distribution of the nuclei by volume. By the 33rd day of the experiment, all the indices studied were almost completely restored to normal.

When γ-AHCS was given after the end of CCl_4 administration (series II), when the necrotic and degenerative changes and the processes of cellular regeneration accompanying them (MI and ILN) had largely subsided, stimulation of regeneration also was observed. The increase in MI and ILN was most marked in the early stages after injection of γ-AHCS. The next day ILN of the hepatocytes was five times higher than that for animals receiving CCl_4 alone. MI was $1.9^{o}/_{oo}$, whereas in animals receiving CCl_4 alone it was 0. In the late stages (23rd, 30th, and 40th days of the experiment) the reaction of the nuclei, in the form of enlargement, was more marked (Fig. 2). γ-AHCS led to normalization of the histological structure of the liver.

The investigations showed that the first reaction to injury of the liver by CCl_4 is an increase in MI and ILN. These parameters changed in most cases in the same direction in the course of the experiment. Later, besides processes of cell proliferation, enlargement of the hepatocyte nuclei and the appearance of cells with larger nuclei, not found in the control, were observed. At different times, sometimes proliferation of the hepatocytes, sometimes their intracellular regeneration predominated. Administration of γ-AHCS against the background of liver damage by CCl_4 stimulates and prolongs regenerative processes, widens their extent, and restores the histological picture to normal. The stimulating action of γ-AHCS is manifested at both cellular and intracellular levels. Just as with the action of

CCl_4 alone, either processes of cell division or an increase in volume of the nuclei predominate at different times. The action of γ-AHCS is phasic in character: alternation of periods of waxing and waning of regenerative processes.

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CHANGES IN THE LIVER FOLLOWING PENETRATION BY A GASTRIC ULCER (EXPERIMENTAL INVESTIGATION)

L. I. Aruin, B. Z. Chikunova, and I. E. Trubitsyna

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Changes in the liver following penetration by a gastric ulcer were studied in 130 albino rats. Ulcers were produced by Okabe's method. During penetration the capsule of the liver is destroyed and four zones can be distinguished in the tissues of the organ: necrosis, demarcation inflammation, necrobiosis, and proliferation of hepatocytes. Together with destructive processes, in the early stages repair processes developed in the parenchyma and connective-tissue structures. Active proliferation of hepatocytes and of bile ducts leads to their penetration into the granulation tissue at the base of the ulcer. After healing of the ulcer complete restoration of the affected areas of the liver takes place but adhesions with the stomach remain.

KEY WORDS: penetration of the liver by an ulcer; regeneration of the liver; proliferation of bile ducts.

Penetration of the liver by a gastric ulcer is found in 2.1% of patients and accounts for about 6% of the complications [4, 5], but the state of the liver itself after penetration by an ulcer has not been studied.

The object of this investigation was to study the course of injury and regeneration of the liver after penetration by experimental gastric ulcers.

EXPERIMENTAL METHOD

Experiments were carried out on 130 albino rats in which chronic gastric ulcers were produced by Okabe's method; the method was described previously [1]. The animals were killed between 1 and 5 months after the procedure. Pieces of liver with adjacent stomach tissue were fixed in 10% buffered formalin and embedded in celloidin and paraffin wax. Sections were stained with hematoxylin-eosin and by Van Gieson's method and impregnated with silver by Foot's method; neutral mucopolysaccharides were detected by the PAS reaction and acid mucopolysaccharides with alcian blue and toluidine blue, RNA was detected by Brachet's method and fibrin by Shueninov's method.

Central Research Institute of Gastroenterology, Main Board for Health Care, Moscow City Executive Committee, Moscow.

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