Relationship between Proliferative and Enzyme Activity of Hepatocytes in 18-Day-Old Rat Pups after Mechanical Injury to the Liver under Conditions of Stimulation with Biogenic Compounds

L. P. Romanova and I. I. Malyshev

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We studied the effect of bioactive compounds Trepel and Suvar on proliferation and enzyme activity of hepatocytes. It was noted that the bioactive compounds activated enzymes in hepatocytes and stimulated their proliferation after mechanical injury to the liver in 18-day-old rats.

Key Words: *hepatocytes; liver mechanical injury; proliferation; enzymes; bioactive compounds*

Under conditions of environmental deterioration, natural zeolites are the factors reducing harmful effects of environmental pollution on humans and animals. Previous studies [1,4,6] showed that they are characterized by a combination of catalytic, detoxification, ion exchange, adsorption, and many other properties. This paved the way for their use in veterinary [9,11] and in medicine [4]. Cationic composition of Alatyr zeolite deposits (Chuvash Republic) favorably differs from other well-studied deposits [4,9]. Their application in veterinary medicine in combination with other immunocorrectors has pronounced growth-stimulating and immunostimulating effects [1,2] and improves hematological and biochemical characteristics of the blood [7] in productive animals.

It was previously demonstrated that bioactive compounds Trepel and Suvar stimulate cell proliferation during healing of mechanical injury of the liver in 18-day-old rats [8]. As a result, the lesion was filled with proliferating hepatocytes over a large area.

Here we studied enzyme activity of rat hepatocytes during healing of liver mechanical injury under conditions of administration of bioactive compounds Trepel and Suvar.

MATERIALS AND METHODS

The study was performed on 18-day-old rats (n=114) weighing 19-28 g. The animals were anesthetized and the liver was pierced through the skin at the right upper quadrant (projection site) with a steel needle 8 cm length and 0.2 cm diameter which had a limiter at a distance of 0.4 cm from the end preventing deeper penetration into tissue; that made it possible to obtain standard injuries. Immediately after surgery, bioactive compounds Trepel (1.25 mg/kg) and Suvar (50 mg/kg) were simultaneously [1,2] added to the basic rat diet. Eighty-nine age-matched rat pups kept after liver puncture on the basic diet served as controls.

The animals were sacrificed with ether on experimental days 1-30. To confirm the presence of trauma and to study restoration and regenerative processes, 1×1 cm fragments were cut out of isolated liver, fixed in 10% neutral formalin and embedded in paraffin. Serial sections were stained with hematoxylin and eosin, by van Gieson, and with iron hematoxylin by Heidenhain. To evaluate liver proliferation, mitoses and binucleated hepatocytes were counted per 7000

Department of Pathological Anatomy, I. N. Ulianov Chuvash State University, Cheboksary, Russia. ***Address for correspondence:*** Malichev_med@mail.ru. Malyshev I. I.

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cells at ×900. To determine the level of enzyme activity, histochemical reactions were performed in hepatocytes. SDH, NADH, NADPH were assessed by the tetrazolium test [5] followed by their quantification; acid phosphatase was detected by simultaneous coupling with naphthols AS phosphates and stable diazonium salts. Quantitative evaluation of enzyme activity in hepatocytes was performed by photometry on Micromed microscope with photographic attachment type FMEL-1 and FEU-79 at the output voltage of the amplifier 1200 V. To obtain monochromatic light beam in the red band passing through the slide, interference filter was used with a maximum transmittance at a wavelength of 620 nm. Light transmission was recorded using a digital voltmeter Schch-4300 followed by taking the negative decimal logarithm; light transmission was transformed into light absorption, which was expressed in units of optical density. The optical density was calculated by the formula: Lg Ui/100, where Ui, voltmeter reading.

Statistical processing of numerical data was performed by Statistica soft using the software package Microsoft Office (Word and Excel).

RESULTS

On postoperation day 1, in control animals slit-like defect of hepatic tissue filled with erythrocytes and dying hepatocytes was seen. Degenerative changes in hepatocytes were observed in the preserved liver tissue and necrobiotic changes at the boundary with the site of injury. On day 2, a large number of inflammatory cells appeared around the injury site. On day 3 and especially on days 5 and 7, the gradual cleansing of the injury site from erythrocytes and dead tissue occurred; by day 7, lymphoid cells were almost completely substituted by fibroblast-like elements. On day 7, thin fibers appeared at the periphery of the damaged area; the amount of fibrous structures at the injury site further increased and on day 11 in the most cases mature fibrous connective tissue was detected at the site of injury. At later terms (days 15, 20 and 30), histological study of the liver revealed foci of fibrous connective tissue almost identical in size.

On days 2-3, a small amount of inflammatory cells appeared around the injury site in experimental animals. On day 9, few fibroblasts were seen. Starting from day 3, injury zone gradually became free from necrotic cells and decreased in size; on day 9 and especially day 11, fibers appeared at the periphery of the lesion. On days 15-20 and later, small area of mature fibrous connective tissue was observed at the site of lost liver tissue.

Thus, in rats of the experimental group reactive exudative reaction around the lesion was less pronounced. The number of fibroblasts in the cell infiltrate was low and they appeared later. This explains the fact that in experimental animals the connective tissue at the site of injury developed later and the focus of fibrosis was visually much smaller.

After injury, intensive mitotic division of hepatocytes was observed in the preserved liver tissue (Table 1). In the experimental rats, the intensity of mitotic division was significantly higher than in the control; in addition, mitoses began earlier and finished much later.

As mitotic activity hepatocyte decreased, the number of binucleated cells in the liver began to increase (Table 2). In animals of experimental and control groups, the number of binucleated hepatocytes increased from experimental day 7; their number was significantly higher in the experimental rats.

The content of acid phosphatase increased (Fig. 1), which can be regarded as the consequence of structural liver disintegration. On the postoperation day 1, the increase was minor and on days 3-11 days enzyme

TABLE 1. Hepatocyte Mitoses (‰) in Experimental and Control Groups (*M*±*m*)

Time after surgery, days	Control	Experiment
1	—	1.4±0.3
3	3.1±1.5	3.6±0.7
5	4.5±2.1	6.4±2.8*
7	3.8±1.9	6.3±2.6*
9	0.9±0.4	5.6±2.7*
11	—	4.5±3.4
15	—	0.8±0.1

Note. *$p<0.001$ in comparison with the control.

TABLE 2. Binucleated Hepatocytes (‰) in Experimental and Control Groups (*M*±*m*)

Time after surgery, days	Control	Experiment
1	16.2±2.4	15.6±2.8
3	16.4±2.1	17.3±2.8
5	17.2±3.0	17.3±2.3
7	28.2±4.6	30.9±6.3
9	29.1±5.4	31.7±4.5
11	30.9±6.5	39.1±6.3*
15	28.2±4.1	40.7±6.5*
20	32.6±3.6	37.2±4.6
30	28.2±2.6	38.1±4.1*

Note. *$p<0.001$ in comparison with the control.

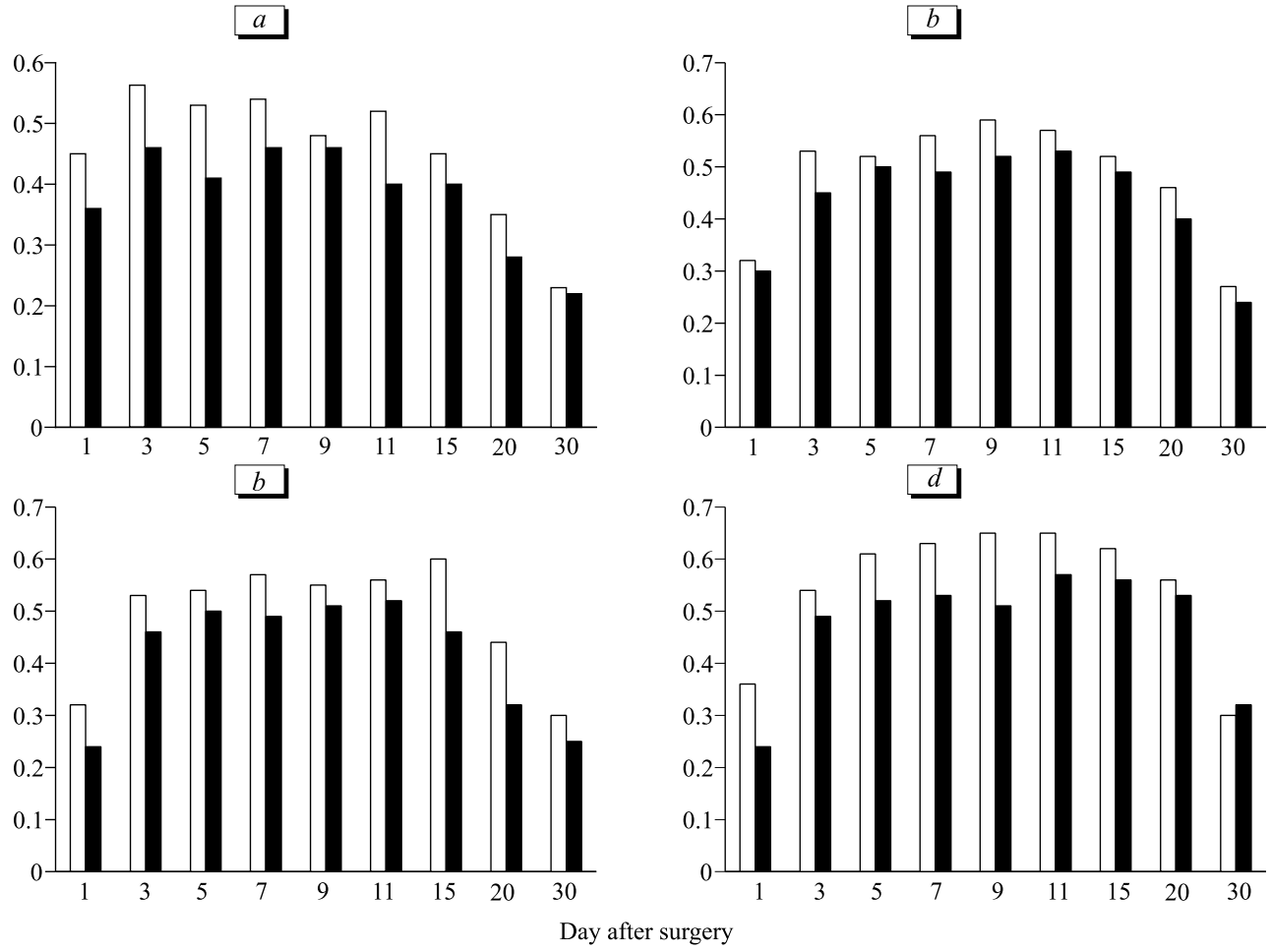

Fig. 1. Activity of acid phosphatase (*a*), SDH (*b*), NADH (*c*) и NADPH (*d*) in hepatocytes of 18-day-old rats in experimental (light bars) and control (dark bars) groups.

activity increased significantly. Starting from post-operation day 3, activity of redox enzymes markedly increased. Enzyme activity remained elevated up to day 20 and then decreased. Overall, the results are consistent with published reports [3,10].

Thus, bioactive compounds Trepel and Suvar provide favorable conditions for healing of liver mechanical injury in 18-day-old rats. In particular, they boost enzyme activity of hepatocytes, which probably underlies intensification of hepatocyte proliferation. Against the background of weakly expressed inflammatory response and delayed collagen formation, actively dividing hepatocytes mechanically compress the injury site, and therefore connective tissue developing there occupies much smaller area than in the control group.

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