MORPHOLOGY AND PATHOMORPHOLOGY

Dynamics of Hepatocyte Proliferation in Regenerating Fetal Rat Liver

A. V. Elchaninov and G. B. Bolshakova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 3, pp. 352-355, March, 2011
Original article submitted January 13, 2010

We studied hepatocyte proliferation in the regenerating liver of 17-day fetuses of outbred albino rats. The animals were sacrificed every 3 h over 2 days after resection of 20% liver. The number of mitoses beyond the zone of injury increased sharply 12 and 24 h after resection. The hepatocyte mitotic index in the perifocal zone did not surpass the mitotic index in regions distant from the focus of injury during any of the studied periods. No circadian rhythm of hepatocyte mitotic activity was detected for resected or intact fetal liver. The injury caused virtually no changes in hepatocyte mitosis phase ratio in the operated compared to intact liver, which attested to stable course of mitosis. The weight of fetal liver recovered at the expense of enhanced mitotic activity of hepatocytes in the entire liver.

Key Words: *regeneration; liver; prenatal period; hepatocyte proliferation; circadian rhythm*

We previously showed that the liver of a 17-day rat fetus restored its weight after 20% excision of the liver tissue within 2 days, presumably due to mitotic division of hepatocytes [3]. Here we studied in detail mitotic activity of hepatocytes during this period, its role in recovery of fetal liver weight, and cell proliferation processes in regions adjacent to the wound during regeneration of fetal liver.

MATERIALS AND METHODS

In ether-narcotized 17-day albino rat fetuses, resection of 20% liver was carried out as described previously [3]. Operated and control fetuses were sacrificed every 3 h over 2 days after liver resection. Intact rats from the same litter served as the control. Experimental and control groups consisted of 7-10 animals. A total of 200 fetuses were studied. Manipulations on laboratory animals were carried out in accordance with bioethics philosophy, regulations of laboratory studies, and ethical standards. Experiments were approved by Bioethics Committee of Institute of Human Morphology.

The liver was weighed, fixed in Carnoy's fixative, and routine histological processing was carried out. Paraffin sections (5 μ) were stained with hematoxylin and eosin.

For evaluation of hepatocyte proliferation, their mitotic index (MI) was determined, expressed in promille (‰). Mitoses were counted per 6000 cells for each animal. MI was calculated separately for the perifocal zone and the area distant from the focus of injury.

Evaluation of mitoses included evaluation of mitosis phase, percent of each phase from all mitoses, and its index for all studied hepatocytes expressed in ‰; stability of the time of hepatocyte mitosis was evaluated from these data. It is known that stability of

Laboratory of Growth and Development, Institute of Human Morphology, the Russian Academy of Medical Sciences, Moscow, Russian. ***Address for correspondence:*** elchandrey@yandex.ru. A. V. Elchaninov

0007-4888/11/1513-0374

the ratio of mitosis phases indicates stability of mitosis duration [2].

The results were processed statistically. The means and standard error of the mean were evaluated by formulas for fractions. The 95% confidence intervals were evaluated using φ test. Comparisons of two selected fractions were carried out using *Z* test. Proportions of mitosis phases were evaluated using χ^2 test [5]. Correlations were detected using Spearman correlation coefficient. The differences were considered significant at 5% significance level. The data were analyzed using Sigma Stat 3.5 software (Systat Software, Inc.).

RESULTS

Hepatocyte MI in resected liver in zones distant from the trauma and in the focus of injury 6 h after resection was significantly lower than in the control ($p<0.001$, $p=0.037$, respectively; Fig. 1). Hepatocyte MI in the focus of injury was significantly lower than beyond it over 24 h after surgery (Fig. 1). Starting from 30 h after the intervention, hepatocyte MI in the perifocal area statistically did not differ from MI beyond this zone.

A strong positive correlation ($r=0.78$, $p<0.005$) was found between hepatocyte MI in the zone adjacent to the wound and outside it. This seemed to indicate that the parenchyma did not grow much from the wound surface during regeneration of fetal liver in rats.

Nine hours after resection, mitotic activity of hepatocytes outside the focus of injury increased and did not differ from this parameter in intact liver ($p>0.003$), while mitotic activity of hepatocytes in the zone adjacent to the wound remained below the control ($p=0.009$). MI of hepatocytes beyond the focus of injury 12-36 h after resection in experimental was higher than in controls. Hence, proliferation in the regenerating fetal liver was stimulated sooner than proliferation in the liver regaining its weight during the postnatal period. For example, the increase in hepatocyte MI in 5-day-old rats was observed 20 h after liver resection [1]. That was presumably due to more rapid reduction of the expression of proliferation inhibitors C/EBPα and p21 after hepatectomy in fetuses compared to older animals [8].

Fifteen hours after resection, MI of hepatocytes outside the zone of injury significantly decreased compared to that 12 h postresection ($p<0.001$), while 18-24 h postresection it significantly increased ($p<0.001$) compared to that 15 h postresection. MI of hepatocytes in the zone adjacent to the wound 12, 15, and 18 h after resection did not differ from the control, while after 24 and 30 h the number of dividing hepatocytes in the zone of injury was significantly higher ($p=0.019$ and $p=0.028$, respectively). Hence, parenchyma in the zone adjacent to the wound started proliferation somewhat later than the hepatocytes in zones distant from the site of injury. After 36 h, hepatocyte MI beyond the focus decreased significantly in comparison with 24 h postresection ($p<0.001$), hepatocyte MI in the focus 36 and 39 h after resection was higher than in the control ($p<0.001$ and $p=0.042$, respectively). Later, mitotic activity of hepatocytes gradually decreased in the zone adjacent to the wound and in zones distant from it. Hence, two peaks of mitotic activity of the regenerating liver hepatocytes were recorded: 12 and 24 h after surgery (14.0±0.6 and 16.1±0.6‰, respectively). The second MI peak was significantly higher than the first one ($p=0.014$). The presence of hepatocyte MI peaks indicated high activity of proliferation [7].

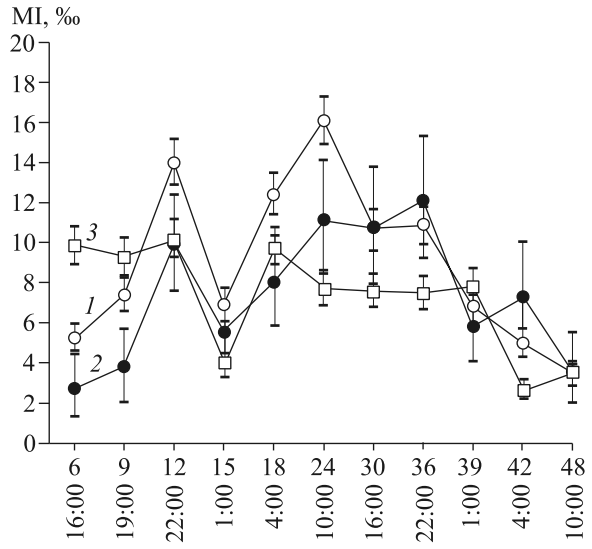

Fig. 1. Hepatocyte MI in the zone adjacent to the wound, in zones distant from the wound, and in intact control throughout 48 after fetal liver resection. Abscissa: upper line: time after resection, hours, lower line: time of the day. *1*) MI outside the zone of injury; *2*) MI in the focus of injury; *3*) intact control.

In the control, mitotic activity of hepatocytes gradually decreased from 6 to 48 h after surgery. This decrease in MI was statistically significant 12-15 and 36-42 h after the intervention.

The decrease in mitotic activity of hepatocytes in the experiment and control 15 h after the operation (01:00) was observed just once and did not repeat after 24 h. Hence, no circadian rhythm of hepatocyte proliferation in the regenerating liver was detected, which was in good agreement with previous data, according to which circadian rhythm of proliferation in rat liver was established during the postnatal period [4,6].

Six hours after the intervention, the percentage of prophases in the operated liver beyond the focus of injury was statistically lower, while that of anaphases higher than in the control ($p<0.05$). Twelve hours

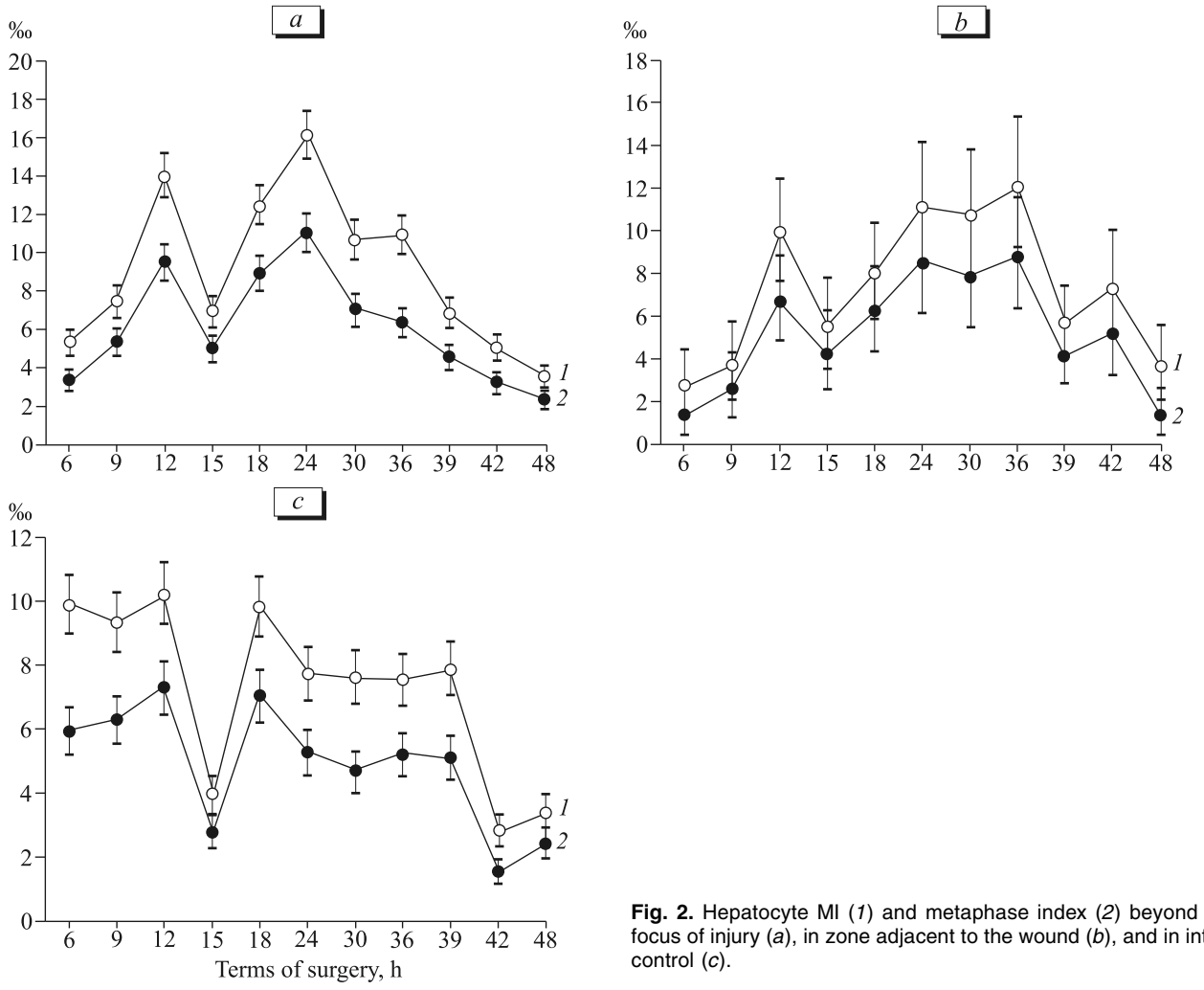

Fig. 2. Hepatocyte MI (*1*) and metaphase index (*2*) beyond the focus of injury (*a*), in zone adjacent to the wound (*b*), and in intact control (*c*).

after the intervention, the percentage of prophases and anaphases in zones distant from the site of injury was statistically higher, while the percentage of telophases was lower than in intact animal liver ($p<0.05$). Moreover, the number of prophases in the focus of injury was also significantly higher than in the control ($p<0.05$). After 24 h, the ratio of mitosis phases changed so that the percentage of telophases in the regenerating liver beyond the perinecrotic area was significantly lower than in the control ($p<0.05$). After 36 h, the percentage of hepatocyte telophases beyond the zone of injury was significantly higher than in the control, while the percentage of metaphases was lower than in the focus and in control animals ($p<0.05$), the level of anaphases in the focus being lower than in the control ($p<0.05$). Later (39 h after resection), the level of prophases in zones distant from the injury was statistically lower than in the control ($p<0.05$).

Metaphases were most incident (Fig. 2) among dividing hepatocytes in the focus, outside it, and in the control, which was explained by the longest duration of this phase in comparison with other phases of mitosis in these cells. The percentage of metaphases at different terms after surgery varied from 50 to 70% (Fig.

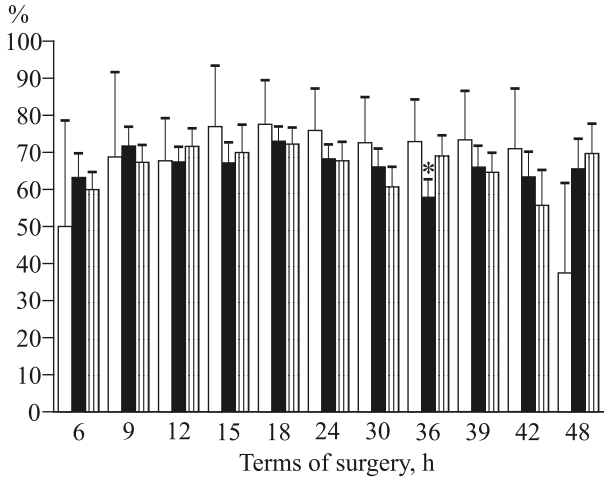

Fig. 3. Percentage of hepatocyte metaphases in the zone adjacent to the wound (light bars), beyond the zone of injury (dark bars), and in intact liver (vertically hatched bars). *$p<0.05$ compared to age-matched controls.

3). Analysis of correlations revealed a strong positive correlation between hepatocyte MI and metaphase index in all cases: near the site of resection, outside this zone, and in the control groups (r=0.97, p<0.05; r=0.96, p<0.05; and r=0.90, p<0.05, respectively).

Opposite shifts in the percentage of hepatocyte mitotic phases, the absence of statistically significant differences between the numbers of metaphases in the zone adjacent to the wound, beyond this zone, and in the control during the majority of periods of the study, and the positive correlation between the metaphase index and MI attest to similar duration of hepatocyte mitosis in the studied zones of the resected and intact fetal liver.

Hence, mitotic division of hepatocytes in zones distant from the injury seems to play the key role in regeneration of fetal liver without appreciable growth of the parenchyma from the wound surface. No circadian rhythm of mitotic activity of hepatocytes was detected in the resected and intact fetal liver. In addition, the injury caused no shifts in the duration of hepatocyte mitosis in fetal liver.

REFERENCES

1. A. G. Babaeva, *Regeneration: Facts and Prospects* [in Russian], Moscow (2009).
2. G. B. Bolshakova, *Byull. Eksp. Biol. Med.*, **145**, No. 4, 471-474 (2008).
3. A. V. Elchaninov and G. B. Bolshakova, *Ibid.*, **150**, No. 9, 352-355 (2010).
4. V. B. Zakharov, S. N. Smirnov, S. G. Mamontov, and E. T. Zakharova, *Ibid.*, **139**, No. 5, 585-589 (2005).
5. V. I. Yunkerov and S. G. Grigoryev, *Mathematical Statistical Processing of Results of Medical Studies* [in Russian], St. Petersburg (2002).
6. H. Barbason, F. Fourre, and C. Focan, *Pathol. Biol.* (*Paris*)., **51**, No. 4, 210-211 (2003).
7. L. Lambotte, A. Saliez, S. Triest, *et al.*, *Am. J. Physiol.*, **273**, No. 4, Pt. 1, G905-G912 (1997).
8. N. A. Timchenko, *Trends Endocrinol. Metab.*, **20**, No. 4, 171-176 (2009).