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# Age-Related Features of the Early Period of Liver Regeneration after Partial Hepatectomy in Rats

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Regenerative processes in the liver were studied in 3-month-old (young) and 9-month-old (aged) male Wistar rats on day 1 after 30 and 70% hepatectomy. Regardless of the resected liver volume, shifts in the biochemical parameters of the serum in aged rats were more pronounced than in young animals. After 30% hepatectomy, no age differences in the rate of hepatic regeneration were found, while after 70% liver resection this parameter was higher in young rats. Hepatectomy in young rats led to recruitment of MSC, hepatocyte precursors, endothelial and epithelial progenitor cells into the liver parenchyma and increased fluidity of the plasma and mitochondrial membranes of hepatocytes. In aged rats, the recruitment of MSC, hepatocyte precursors, and endothelial progenitor cells into the injured liver was impaired and the rigidity of the mitochondrial membranes of hepatocytes increased.

**Key Words:** *liver resection, age-related features; stem and progenitor cells; hepatocytes; membrane microviscosity*

The liver has a unique ability to regenerate and restore its functions after injury, surgical resection, or toxic damage. In most patients, about 30% of the initial size of the organ is sufficient for successful restoration of liver functions after injury or resection (in patients with concomitant parenchymal hepatic diseases 40-50%) [1]. Posthepatectomy hepatic failure is a serious complication after liver resection developing in 32% of cases [2]; this condition is one of the main factors of postresection mortality [1].

The lost hepatic cells are replenished via cell division, *e.g.*, hepatocytes form new hepatocytes, cholangiocytes form cholangiocytes, and the same pattern is observed for hepatic stellate cells, endothelial cells, Kupffer cells, and other cell types [3]. Another option for repairing damage at the cellular level is transdifferentiation between different types of hepatic cells. Studies involving rats, patients, and fish *Danio rerio* showed that hepatocytes and cholangiocytes can play a role of progenitor cells by passing through the dedifferentiation stage followed by differentiation into other types of cells necessary to restore lost functions [3]. There is evidence that regeneration involves not only resident cells, but bone marrow and hepatic stellate cells (Ito cells) [4]. Endothelial bone marrow progenitor cells participate in the regeneration of the hepatic endothelium, and the capability of mesenchymal stem cells (MSC) for the multilinear differentiation suggests their possible participation in the regeneration of liver parenchyma [5].

Among the main factors affecting the hepatic regeneration ability, age and resection volume are most interesting due to the scarce coverage [6]. So, it is still

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unknown what mechanisms preserve the main hepatic functions at the same level, despite the age-related decrease in liver volume by 20-40% [7]. On the other hand, it is not clear what is the cause for insufficiency or failure of regeneration initiation in some aged patients.

Hepatocyte membrane deserves special attention, because modifications of the plasma and mitochondrial membranes are thought to be implicated in body aging and age-related diseases. Membrane fluidity and activity of membrane-associated enzymes decreases with age, which results in reduced production ATP and intensification of redox stress [8]. However, the exact mechanisms underlying changes in the fluidity of the mitochondrial membrane and their age-related features during regeneration remain unknown. There are no effective approaches to stimulate hepatocyte regeneration after injury or liver resection and practices for the treatment of postectomy liver failure in aged patients.

Our aim was to study the response of stem and progenitor cells and plasticity of hepatocyte membranes in rats of different ages after partial liver resection.

## MATERIALS AND METHODS

The experiments were performed on male Wistar rats obtained from the nursery of the Department of Experimental Biomodeling of the E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine (veterinary certificate is available). All manipulations were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasburg, 1986). The study was approved by the Ethics Committee of the E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine (Protocol No. 107a032020; May 5, 2020).

The animals were divided into 6 groups (5 animals in each group): intact 3-months-old (young) and 9-months-old (aged) rats (groups 1 and 2), young and aged rats with 30% liver resection (groups 3 and 4) and 70% liver resection (groups 5 and 6). The rats aged 3 and 9 months at the beginning of the experiment.

Partial resection was performed as described elsewhere [9]. The rats were subjected to 30 or 70% liver resection; anesthesia was performed by isoflurane inhalation using a Ugo Basiele 21050 inhalation anesthesia machine.

The rate of liver regeneration (RR) was calculated by the formula:

$$RR=\frac{(LW_m/100\ g\ BW)_{sac}}{(LW_p/100\ g\ BW)_{res}}\times 100\%,$$

where LWm is the measured liver weight at sacrifice (sac); LWp is the preoperative (pre) projected liver weight, BW is the body weight [10].

The content of stem and progenitor cells in the liver was assessed by flow cytometry using specific antibodies to surface markers CD45 (554878; BD), CD90 (751181; BD), CD133 (ab19898; Abcam), CD326 (ab282457; Abcam), OV6 (MAB2020-SP; R&D Systems) on a FACS Canto II flow cytometer with FACSDiva software (BD Biosciences).

The mitochondria were isolated by differential centrifugation. The microviscosity of the plasma and mitochondrial membrane of hepatocytes was assessed by measuring lateral diffusion of hydrophobic fluorescent pyrene probe and calculating pyrene eximerization coefficients $K=I_{470}/I_{390}$ at $\lambda=340$ nm for the lipid bilayer and $\lambda=285$ nm for the lipid-protein zones. The method is based on the formation of pyrene excimers (active dimers) in the lipid environment. The eximerization coefficient inversely depends on microviscosity [11]. Pyrene fluorescence was measured on The Cary Eclipse (Varian) fluorescence spectrometer.

The levels of total and direct bilirubin, alkaline phosphatase, ALT, AST, cholesterol, triglycerides, LDL, HDL, and glucose in the blood serum were measured by biochemical methods.

Statistical analysis was performed by methods of variational statistics using the SPSS Statistics 12.0 software (IBM). The arithmetic mean ($M$), error of the mean ($m$), and the probability value ($p$) were calculated. Significance of differences was assessed using the nonparametric Mann–Whitney $U$ test and Wilcoxon's test for linked samples. The difference between the two compared values was significant at $p<0.05$.

## RESULTS

Shifts in biochemical parameters of the blood serum were found in rats of both age groups on day 1 after 30% liver resection. In aged rats, the increase in the levels of alkaline phosphatase and direct bilirubin and the decrease in cholesterol and HDL levels were more pronounced than in young animals ($p<0.05$; Table 1). At the same time, hyperglycemia and elevation of LDL levels ($p<0.05$) were detected in young rats, but not in aged animals. Simultaneously with changes in biochemical parameters, regeneration processes were initiated in rats of both age groups on day 1 after 30% liver resection (Fig. 1); no age-related differences in the rate of regeneration were detected.

According to modern concepts, stem cells (SC) and progenitor cells are implicated in regeneration of damaged tissues and organs [11]. We do not exclude age differences in the response of SC to partial resection in male Wistar rats. Flow cytometry

**TABLE 1.** Biochemical Parameters in the Blood Serum of Young (3 months) and Aged (9 months) Male Wistar Rats after 30 and 70% Liver Resection, on the First Day of the Experiment (*M*±*m*)

| Parameter | Young | | | Aged | | |
|---|---|---|---|---|---|---|
| | intact | 30% resection | 70% resection | intact | 30% resection | 70% resection |
| Alkaline phosphatase, U/liter | 103.99±7.26 | 159.95±19.80* | 232.88±20.40* | 87.53±3.09 | 208.34±23.14* | 242.88±55.46* |
| ALT, U/liter | 51.59±3.27 | 171.58±56.38* | 654.63±415.64* | 53.75±3.26 | 785.58±587.38 | 97.13±19.80 |
| AST, U/liter | 182.24±10.53 | 659.28±273.79* | 1472.38±955.70* | 125.23±9.55$^{o}$ | 1129.08±821.12 | 238.25±40.49$^{o}$ |
| Bilirubin, μmol/liter total | 0.60±0.03 | 1.70±0.32 | 3.60±0.81 | 0.60±0.24 | 5.2±2.3 | 4.10±0.59 |
| direct | 0.48±0.10 | 0.53±0.05 | 0.90±0.23 | 0.60±0.07 | 1.50±0.62$^{o}$ | 1.12±0.14* |
| Cholesterol, mmol/liter | 2.74±0.25 | 1.66±0.12* | 1.42±0.10* | 2.88±0.40 | 1.67±0.10* | 1.71±0.12* |
| Triglycerides, mmol/liter | 0.91±0.09 | 0.70±0.03 | 0.6±0.1 | 1.52±0.21$^{o}$ | 1.07±0.30 | 0.88±0.20 |
| Glucose, mmol/liter | 5.71±0.48 | 7.41±0.17* | 8.50±0.79 | 6.56±0.13$^{o}$ | 7.73±0.53 | 6.81±0.23$^{o}$ |
| HDL, U/liter | 1.04±0.07 | 0.67±0.06* | 0.55±0.02* | 1.27±0.17 | 0.64±0.08* | 0.68±0.04*$^{o}$ |
| LDL, U/liter | 0.55±0.04 | 0.73±0.04* | 0.70±0.08* | 0.87±0.18$^{o}$ | 0.75±0.08 | 0.78±0.13 |

**Note.** $p<0.05$ in comparison with *age-matched intact control (Mann—Whitney *U* test), $^{o}$young animals of the corresponding group (Mann—Whitney *U* test).

of surface antigens revealed a number of common patterns. In particular, 30% liver resection caused a significant increase in the content of $CD45^{-}CD326^{+}$ and $CD45^{-}CD326^{+}CD133^{-}$ epithelial cells (EC), $CD45^{-}CD326^{+}CD133^{+}$ and $CD326^{+}CD133^{+}CD90^{+}$ epithelial precursors of hepatocytes (EPH) and MSC in young rats relative to intact controls ($p<0.05$; Table 2). In aged rats, 30% resection also increased the number of EC, but the number of EPH, on the contrary, decreased ($p<0.05$; Table 2).

The presented data confirm the assumption [5] about the participation of resident (epithelial progenitor cells and EPH) and non-resident SC (MSC) in the restoration of lost hepatic structures of young rats after 30% liver resection. The absence of a generalized cellular response to resection in aged rats can be explained by initially higher number of precursors in comparison with young rats. According to our data, intact aged rats have significantly ($p<0.05$) higher numbers of MSC (by 1.62 times), $CD45^{-}CD326^{+}$ EC (by 7.41 times), $CD45^{-}CD326^{+}CD133^{-}$ EC (by 5 times), $CD45^{-}CD326^{+}CD133^{+}$ EPH (by 5.52 times), $CD45^{-}CD326^{+}CD133^{+}CD90^{+}$ EPH (by 2.2 times), $CD45^{-}CD133^{+}CD90^{-}$ endothelial cells (by 16.79 times) than intact young rats (Fig. 2). The initially high number of resident and non-resident SC can have a positive effect on liver regeneration in aged rats (Fig. 2). After partial liver resection, we revealed infiltration of the hepatic parenchyma by $CD45^{hi}$ lymphocytes in aged rats, but not in young animals (Table 2). Previous study has shown that the sensitivity of SC to oxidative stress and inflammation decreases with age [12]. It is highly probable that the decrease in the content of hepatocyte precursors revealed in our study in aged rats after 30% liver resection can be due to the inhibitory effect of inflammation (Fig. 1).

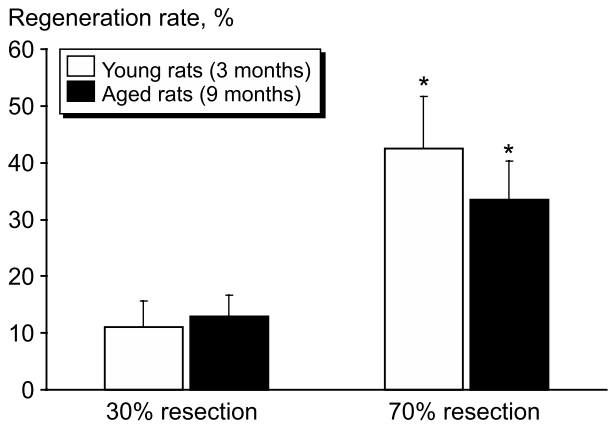


**Fig. 1.** The rate of liver regeneration on the first day after 30 and 70% liver resection in young and aged male Wistar rats. *$p<0.05$ in comparison with 30% liver resection in rats of the corresponding age (Mann—Whitney *U* test).

Liver regeneration is associated with compensatory hyperplasia and hypertrophy of hepatocytes, the mechanisms of which are relatively well characterized and plasticity of membranes play an important role in these processes. According to some data, aging is associated with reduced membrane fluidity and reduced activity of membrane-associated enzymes, which determines impaired ATP production and increased redox stress [8]. However, the mechanisms of age-related changes in the fluidity of plasma and mitochondrial

**TABLE 2.** Effect of 30 and 70% Liver Resection on the Content of Cells in the Parenchymal Fraction of Cells Isolated from the Liver of Young and Aged Male Wistar Rats on the First Day of the Experiment (*M*±*m*)

| Cells | 30% resection | | 70% resection | |
|---|---|---|---|---|
| | young | aged | young | aged |
| EC | 1054.27±82.81* | 277.11±23.94*+ | 3571.76±129.90* | 72.65±52.03+o |
| CD133⁻ EC | 958.90±56.49* | 397.11±39.17*+ | 3337.57±112.99* | 96.17±59.65+o |
| EPH | 1358.62±158.92* | 67.46±1.98*+o | 5675.12±1.49* | 40.40±7.69*+o |
| CD90+ EPH | 382.46±28.61* | 17.54±4.59*o | 3277.63±1000.08* | 44.91±66.15o |
| Endothelial cells | 111.92±27.46 | 98.06±11.88*+o | 208.98±18.14* | 52.18±3.93+o |
| Oval cells | 106.04±0.70* | 96.98±49.20o | 459.45±36.86*+ | 121.15±23.35o |
| MSC | 156.50±5.52* | 117.85±0.15* | 235.14±15.25*+ | 75.13±25.80o |
| Hematopoietic stem cells | 31.43±5.17* | 58.46±13.21+o | 213.33±40.17* | 50.32±3.62*o |
| Lymphocytes | 95.87±17.22 | 355.93±48.84 | 202.07±12.63 | 87.04±15.25 |
| CD45hi lymphocytes | 123.24±28.77 | 258.39±9.45*+ | 282.93±57.00* | 20.88±5.46+ |

**Note.** Cell content in age-matched intact control was taken as 100%. $p<0.05$ in comparison with *age-matched intact control (Wilcoxon's *W* test), +30% liver resection in the corresponding age group (Mann—Whitney *U* test), oyoung animals of the corresponding group (Mann—Whitney *U* test).

membranes of hepatocytes, especially in the early post-traumatic period, remain unknown. In this regard, we evaluated pyrene excimerization factors in the plasma and mitochondrial membranes of hepatocytes on the first day after 30% liver resection. According to our data, the mobility of hepatocyte membranes in the area of lipid layers and protein–lipid contacts in young animals with partial resection increased in comparison with that in intact animals ($p<0.05$; Fig. 3). At the same time, no significant changes in the mitochondrial membranes were found. This state of the membranes is typical of mitotic activity of cells. In this regard, we do not exclude the development of compensatory hyperplasia of hepatocytes in young rats in response to resection. At the same time, the viscosity of the hepatocyte plasma membrane in aged rats increases. It is possible that the high regeneration rate observed in this group, which is not inferior to that of young rats (Fig. 1), is provided by the mechanism of cellular hypertrophy. The implementation of this process requires intensive energy exchange, which is provided by division of mitochondria. The higher fluidity we have found in the area of protein–lipid and lipid contacts of mitochondrial membranes of hepatocytes in aged rats indirectly supports this assumption (Fig. 3).

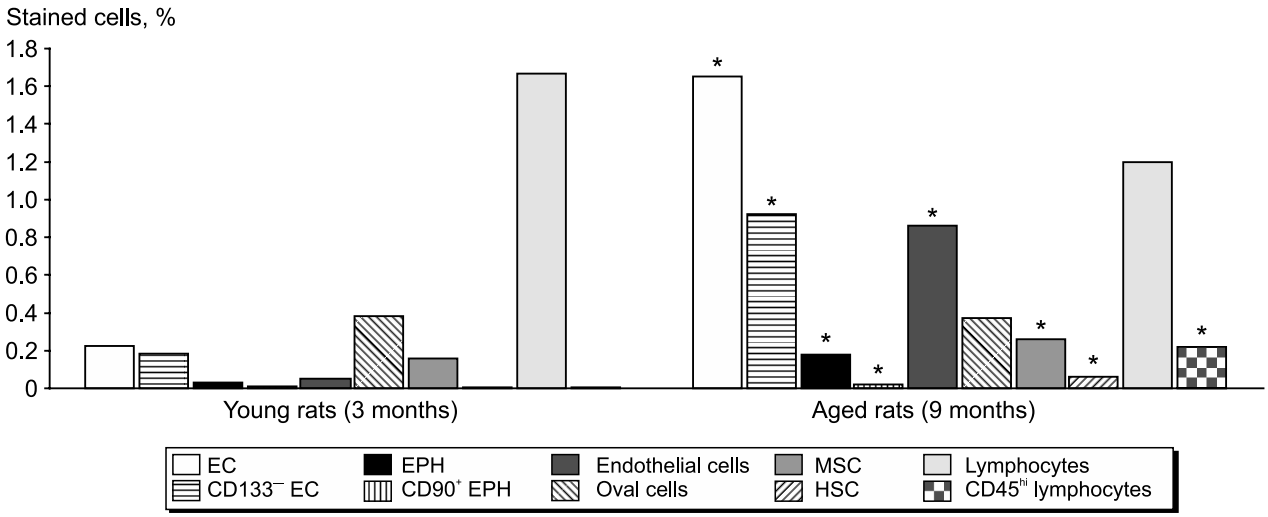


**Fig. 2.** The content of stained cells in the parenchymal fraction of liver cells in intact young and aged male Wistar rats (% of all mononuclear cells). HSC, hematopoietic stem cells. *$p<0.05$ in comparison with young animals (Mann—Whitney *U* test).

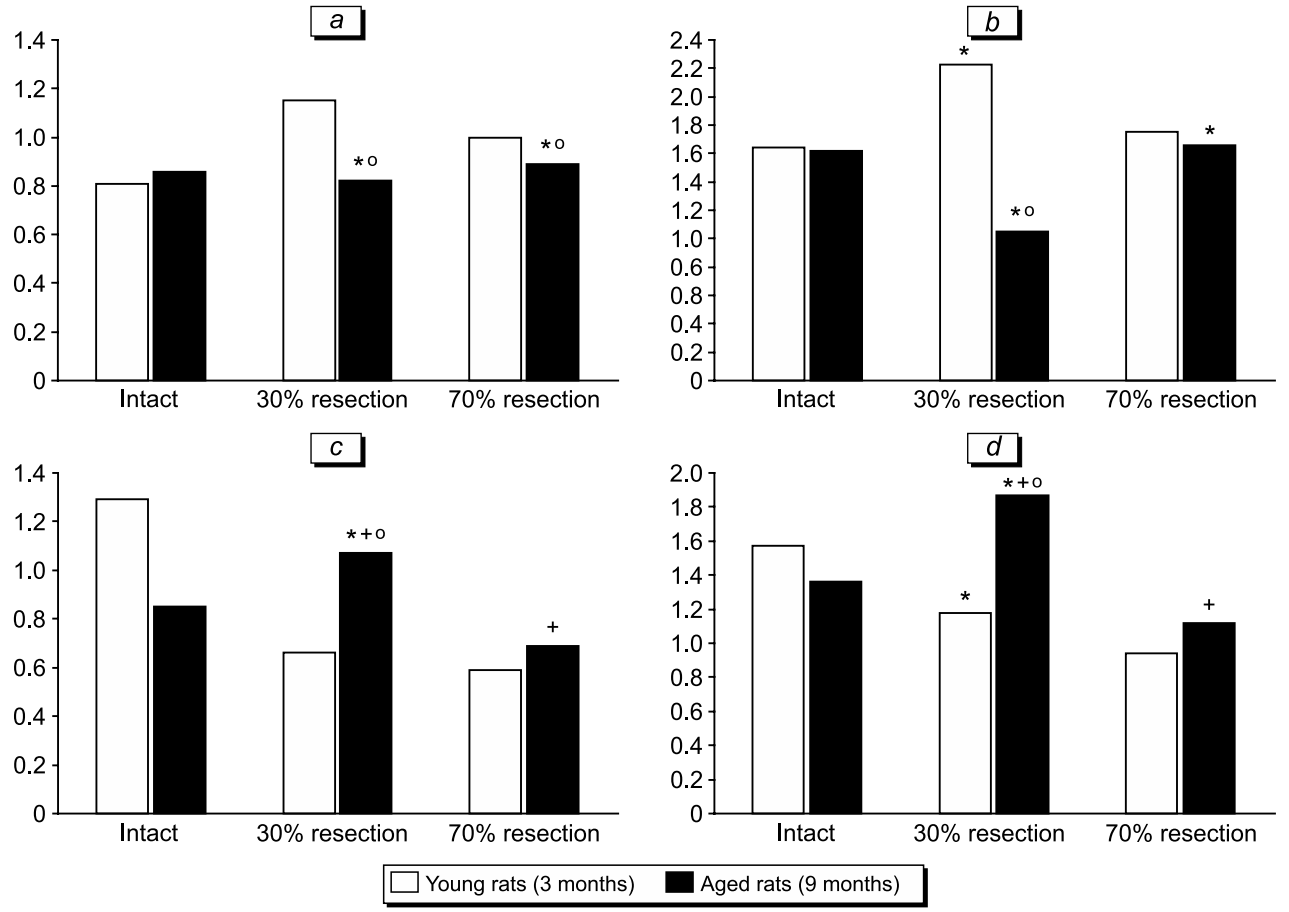


**Fig. 3.** Effect of 30 and 70% liver resection on the pyrene eximerization coefficient in regions of protein—lipid contacts (*a*, *c*) and lipid bilayers (*b*, *d*) of the plasma (*a*, *b*) and mitochondrial (*c*, *d*) membranes of hepatocytes in young and aged male Wistar rats on the first day of the experiment. $p<0.05$ in comparison with *age-matched intact control, $^{+}$30% liver resection in the corresponding age group, $^{o}$young animals of the corresponding group (Mann—Whitney *U* test).

Therefore, the results of this study indicate the existence of age differences in the hepatic regeneration of male Wistar rats with 30% organ resection. The restoration of lost liver cells in young rats with high compensatory capabilities occurs due to the mitotic activity of hepatocytes and involvement of SC and progenitor cells in the process. The higher sensitivity of aged rats to inflammation (due to age-related changes) negatively affects the regenerative potential of precursors and the ability of hepatocytes to proliferate. Therefore, the search for and development of approaches to accelerate hepatic regeneration in elderly patients undergoing partial resection require to specially focus on the selective blockade of inflammatory factors that reduce activity of SC and progenitor cells, and, on the other hand, to develop treatment strategies that increase the plasticity of hepatocytes plasma membrane. The correctness of this therapy is supported by our resultant studies using the model of 70% liver resection in male Wistar rats. In young rats, SC participated in liver regeneration (Fig. 2, Table 2). In aged rats, the number of SC in the hepatic parenchyma was lowered and plasticity of hepatocyte membranes was impaired, which negatively affected the rate of liver regeneration: this parameter in aged animals after 70% liver resection was lower by 27% than in young animals (Fig. 1).

In summary, the hepatic remnant after partial resection is a potentially important object of study, because it allows assessing the regenerative potential of parenchymal SC and to adjust the therapy aimed at accelerating tissue recovery. In a certain course of the disease, autologous cell therapy can solve the problem of regenerative-competent cell deficiency.

**Conflict of interest.** All authors have no conflicts of interest.

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