Histological and Morphometrical Studies in Liver Regeneration in Mice

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Abstract
Liver regeneration after loss of important cell population is a complex process involving all mature liver cell types. We study the response of hepatocytes in 24h and 48h after partial hepatectomy by microscopic and morphometric analysis. Thirty adult male NMRI, 8 week old mice were used in the study. Liver regeneration after partial hepatectomy (68%) in mice is a complex process that is performed on account differentiated mature hepatocyte proliferation, as evidenced by the presence of nuclei hypertrophy, binucleate cells or polyploidy, and numerous mitotic figures. Morphometrical analysis of cell size showed increases in average dimension of the control compared to the experimental groups. After this study, we can conclude that liver regeneration after partial hepatectomy is marked by the proliferation of liver cell population, while reducing the apoptotic process.

Keywords: histology, liver regeneration, morphometry

1. Introduction

The liver has the extraordinary capacity to regenerate after traumas or various injuries (infectious or toxic) [1, 2, 3]. Knowing the pathways and molecules involved in the regeneration of liver tissue will allow us in the future, by applying modern techniques of cellular and molecular biotechnology and genetic engineering, to act upon certain levels or specific molecules, so that we can control the balance between cell proliferation or death. Thus, such knowledge may prove useful in the medical and pharmaceutical industry for liver health issues in humans or animals, both for diagnostic and prognostic purposes (identification of new markers), but also in therapy (use of cell or gene therapies).

On the other hand, with the accelerated world population growth and the increasing demand for food, biotechnology might help solve the control over the de novo regeneration and formation of tissues and organs.

Liver regeneration after significant loss of cell population is a complex phenomenon in which all types of mature hepatic cells participate and is also a process very well orchestrated by many signaling cascades involving: growth factors, cytokines, extracellular matrix remodeling, stimulation and/or inhibition reactions of growth and proliferation signals, molecular pathways of cell death (apoptosis or necrosis) [4, 5]. In adult

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pluricellular organisms, the balance between cell proliferation (division) and apoptosis is determined by genetic adjustment. The precise adjustment and control of cell cycle, namely the balance between proliferation and apoptosis, plays a major role in the normal proliferation and differentiation process. Liver regeneration involves a marked proliferation of liver cell population and also a decrease of the apoptotic process.

The aim of this study is to evaluate the histological injuries in mice liver after partial hepatectomy. The purpose of the morphometric analysis was the quantitative assessment of the nuclei sizes and the comparison of such values with those obtained with the experimental groups.

2. Materials and methods

The material studied is represented by fragments of liver tissue, drawn after the necropsy from 30 male mice, aged 8 weeks, of the NMRI line, weighing 31.22±1.22. The mice were raised in the Cellular and Molecular laboratory of the University of Medicine and Pharmacy Timisoara in standard conditions of humidity (45-55%), temperature (25°C) and light control (12h light/12h dark). The mice were fed normal diet and water ad libitum.

Experimental animal model

The partial hepatectomy (HP) was performed according to the surgical technique of Higgins & Anderson [6], under sterile conditions. Mice were anesthetized by intramuscular injection of Ketamine/Xylazine (100mg/kg Ketamine mixed with 10mg/kg Xylazine). A midline incision was made, from the right side of the breastbone along the xiphoid. After the exposure of the liver, the four major lobes were identified (the right, the median, the left and the caudate) and the two segments of the median lobe were separated from the gallbladder. Subsequently, both right and left lobe and the lateral of the median lobe (68% of the total liver mass) were ligated with 3/0 catgut thread.

Histological study

After 24h and 48h respectively, the animals were sacrified. Tissue fragments were taken from the lobes and fixed in formaline. The samples were cut into pieces and then stained with hematoxylin and eosin. The study animals were divided into 3 groups: group A (control), group B (animals sacrificed at 24h after HP) and group C (animals sacrificed at 48h after HP). The morphometric analysis aimed to measure the size of the nuclei in the control group versus experimental groups.

The target parameters were: diameter, area and perimeter of nuclei, which were determined using the QuikPHOTO MICRO 2.2 program, by means of which the mean values and the standard deviation were obtained. The study included each 10 nuclei per microscope field.

Statistical data interpretation

Data on the tested parameters were selected and entered into computer database tables and into the Microsoft Excel spreadsheed module of the Microsoft Office 2007 software package. The values obtained were expressed as mean value ± standard deviation. The results of the control and experimental groups were compared using the Student test (two samples, unpaired, non equal variance). Interpretation was done by analyzing the p value at the significant level of 0.05 [7].

3. Results and discussion

The microscopic samples were stained to highlight the general and overall appearance with HE staining. The samples obtained from the animals included in the control group (group A) and the microscopy images revealed a normal appearance of parenchyma and liver extracellular matrix – hepatocytes are arranged in cords located between the sinusoidal capillaries and oriented radially to the terminal venula. Hepatocytes have polygonal shapes, most mononucleate. We noted the presence of rare apoptotic bodies and extramedullary hematopietic groups. We found no evidence of pathological lesions: steatosis, necrosis or degenerative changes (Figure 1, 2).

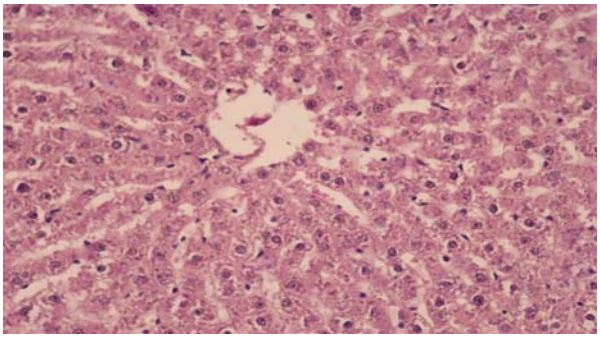

Figure 1. Group A, HE staining, 400X – liver normal structure

In the experimental groups, cell and nuclear changes, as well as structural changes in the liver parenchyma – capillary neoformation, were recorded (Figure 3). In the experimental group B, mice sacrificed at 24h after HP, we noted hypertrophic hepatocytes, binucleated or with large nuclei and prominent nucleoli; some mitoses were also detected (Figure 4, 5).

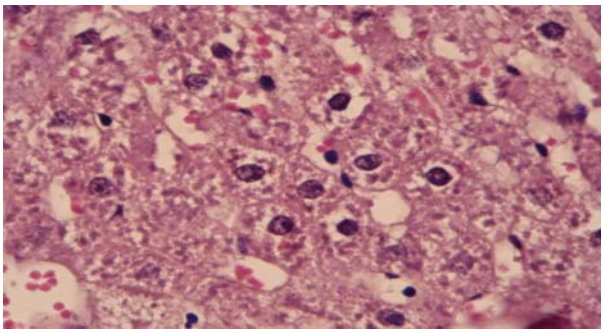

Figure 2. Group A, HE, 1000X, normal liver structure

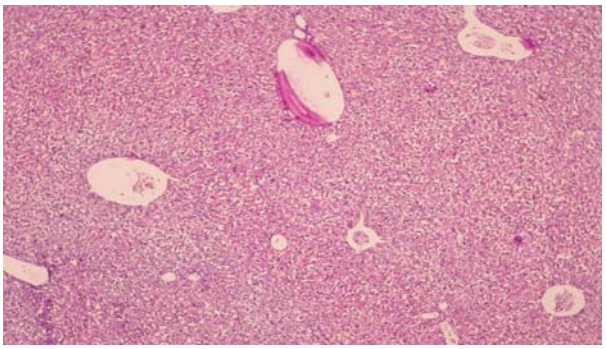

Figure 3. Group B, HE, 100X, capillary neoformation

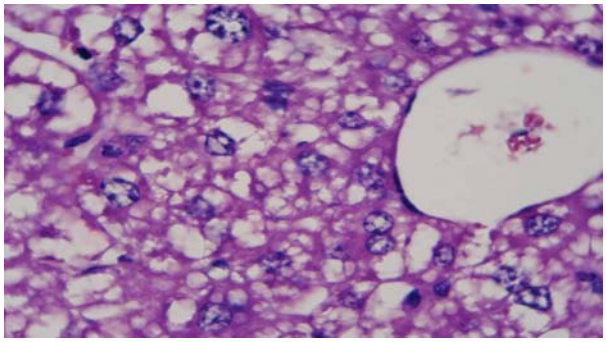

Figure 4. Group B, HE, 1000X, binucleated cells

Also, many optically inactive areas, well-delineated accumulations of fatty vesicles, were present in the cytoplasm (Figure 6).

In group C, mice sacrificed at 48h after HP, the whole group was characterized by macrovesicular steatosis. We noticed many hypertrophic hepatocytes, binucleated or with hypertrophic nuclei, as well as some mitotic figures (Figure 7).

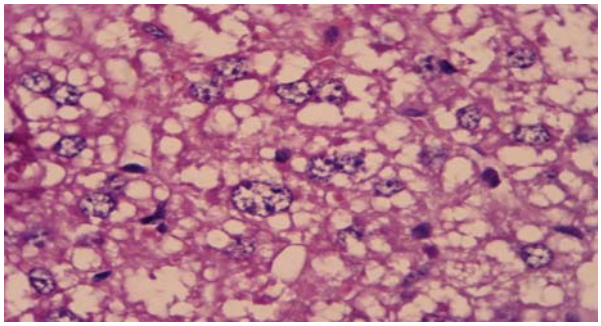

Figure 5. Group B, HE, 1000X, large nuclei

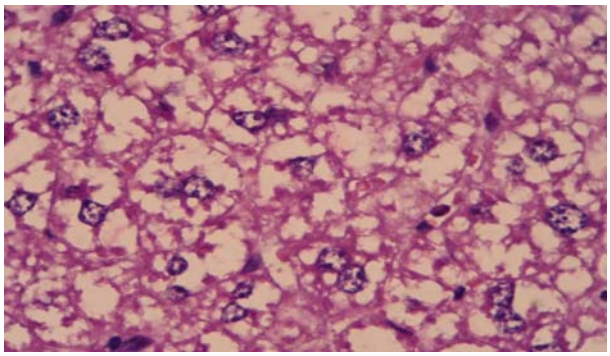

Figure 6. Group B, HE, 1000X, fatty vesicles

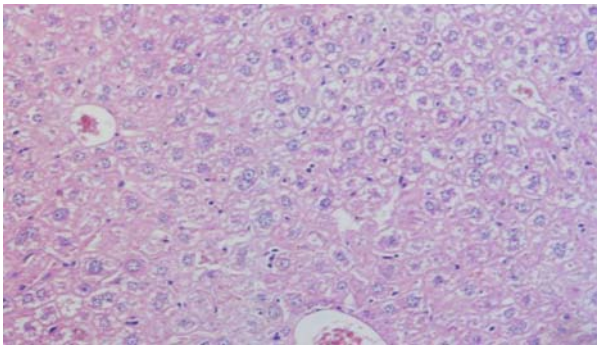

Figure 7. Group C, HE, 400X, mitotic figure, hypertrophic and binucleated nuclei,

Many neoformated vessels were remarked in the cytoplasm. In some areas, especially in the periportal one, the lobular architecture of the liver parenchyma was changed and no evidence of classic hepatocyte cords and hepatic radial sinusoids was seen. The presence of large cube-shaped cells, placed in groups, and sinusoid vessels arranged unorderly was also noticed (Figure 8).

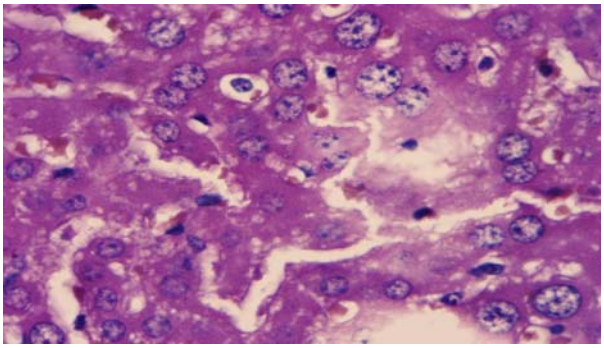

Figure 9. Group C, HE, 400X, hepatocytes with aberrant disposition

The level of nuclear hypertrophy was determined based on the diameter, area and perimeter of nuclei. The results obtained were expressed as mean values ± standard deviation. Comparing the parameters ascertained with the three groups, we observed an increase of the mean values in the experimental groups versus the control group, as well as higher mean values in group C in comparison with group A, for all parameters analyzed (Figure 10, 11, 12).

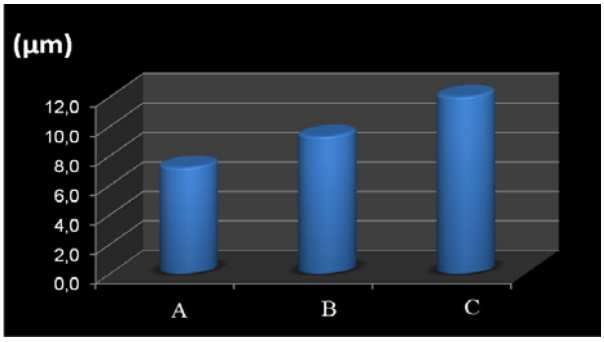

Figure 10. Representation of the mean values of nuclei diameters from the three groups

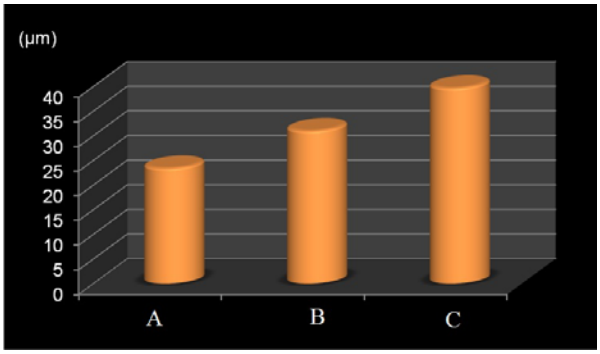

Figure 11. Representation of the mean values of nuclei perimeters from the three groups

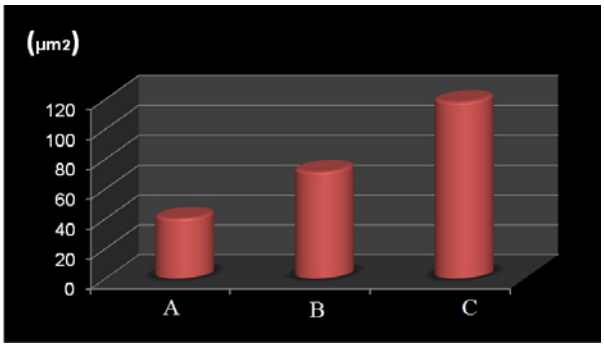

Figure 12. Representation of the mean values of nuclei diameters from the three groups

Although most somatic cells are diploid, somatic polyploidy occurs in almost all mammal tissues, as polyploid cells are present in the myocardium, in the walls of the large blood vessels, trophoblast, nervous tissues, retinal pigment epithelium, kidney and bladder Polyploidy appears only in tissues with proliferation and differentiation capacity, and the mammals hepatocytes have the particularity to contain one or more diploid nuclei with different DNA amounts [8]. Besides the differences between the species, the 4n cell population prevails in the human adult liver, while

during the fetal and postnatal period, the hepatocytes are exclusively 2n. The processes of polyploidization and binucleation are intimately linked and irreversible, and the loss of cell - pluripotency is final [9, 10, 11, 12]. This may be considered as a normal mitotic block.

In G0 and G1 the hepatocytes are diploid, containing 2n (46 chromosomes), but with the genetic material reduced to a half. After the S phase, the DNA amount doubles, and in G2 and M the cells are 4n (tetraploid). Following cytokinesis, 2 daughter cells with 2n chromosomes are divided. When mitosis or cytokinesis does not occur, binucleate cells or cells with tetraploid nuclei are being formed. Partial hepatectomies determine proliferation of all populations in the liver structure, especially hepatocytes. DNA synthesis starts within the first 10-12h after surgery and ends in about 30 days. The cell proliferation begins in the periportal area, near the portal triads, and continues slightly to the center of the hepatic lobules. By proliferation, hepatocytes are initially piled, and then they rearrange themselves quickly, forming the classic cords [13]. The high number of large nuclei, i.e. of polyploids which were noticed, is similar with the data reported in the literature; at 24h after HP the number of polyploid or binucleated cells increase. This confirms that mature hepatic cells are involved in this type of regeneration: mature cells undergo a division process and form daugher cells with higher degrees of ploidy [1]. In a study in rats, Coelho et al. reported similar results to those of our study: in the first day after HP, they revealed vesicular nuclei, prominent nucleoli and hepatocytes in different stages of mitosis. They reported numerous cytoplasmic lipid inclusions that disappeared gradually in the next days, while the mitotic figures increased. In newborn rats, the histological structure of the liver is similar to that tested after 7 days from the HP [14]. The occurrence of macrovesicular steatosis in the early stages is due to the failure of the enzyme system, reduced after HP, to metabolize lipids.

4. Conclusions

After this study, we can conclude that the liver regeneration after partial hepatectomy in mice is a complex process that is performed on account of differentiated mature hepatocyte proliferation, as evidenced by the presence of nuclei hypertrophy, binucleated nuclei, and numerous mitotic figures.

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