DOI: 10.4149/BLL_2024_005

Bratisl Med J 2024; 125 (1)

24 – 32

EXPERIMENTAL STUDY

Use of hepatocyte transplantation after extensive liver resections in experiment

Rastislav KALANIN[1], Tomas GAJDZIK[1], Jana KATUCHOVA[1], Silvia FARKASOVA IANNACCONE[2], Peter JARCUSKA[3], Stefan HULIK[1], David ADANDEDJAN[1], Igor GALA[4], Jozef RADONAK[1]

Ist Department of Surgery, PJ Safarik University, Faculty of Medicine and L Pasteur University Hospital, Kosice, Slovakia. **rastislav.kalanin@unlp.sk**

ABSTRACT

The aim of our research is to prove, that the transplanted hepatocytes can survive and compensate for inadequate liver function in experimental animals. Thesis discusses the basic principles of hepatocyte transplantations, the process of their adaptation and effect on the organism as well as possibilities of their further use as a promising treatment method.In the experiment we used hepatocytes isolated and produced from explanted livers of laboratory rats. Hepatocytes form the basis of liver tissue and are responsible for a number of metabolic processes ocurring in the liver, therefore is the possibility of their use as a cell therapy one of the alternative treatment methods for liver failure.

Into the laboratory experiment, rats of the Sprague Dawleyspecies were placed, which we divided into five groups of equal numbers. Groups consisted of healthy rats, rats after 2/3 liver resection and rats with liver damage induced by intraperitoneal administration of thioacetamide.Two groups with impaired liver had hepatocytes prepared in advance transplanted into their portal vein.Hepatocyte function was assessed in the blood serum and compared with a control group of healthy animals and groups with liver damage without the application of hepatocytes at the same time intervals.Whole experiment lasted 21 days. On the last day of the experiment all the animals were killed by decapitation.Livers of all animals were explanted and sent for histopathological analysis which used classical histological methods. After evaluating the results we observed significant changes of evaluated laboratory levels in blood serum and weight in experimental animals after the transplantation of hepatocytes in comparison with the laboratory results of the control group of healthy animals and groups with liver damage without transplanted hepatocytes. The results of our experiment show that by biochemical and histological analysis we managed to clearly prove the function of transplanted hepatocytes in the model of laboratory rat with impaired liver function *(Tab. 5, Fig. 17 , Ref. 17)*.

Text in PDF *www.elis.sk*

KEY WORDS: liver, transplantation, hepatocytes, liver tumors, resection of liver, liver failure, experiment.

Materials and methods

All experimental conditions of the experiment were in accordance with the European rules of ethical standards of animal care. The experiment was approved by the Ethics Committee of the Faculty of Medicine of the University of PJ Safarik and the State Veterinary and Food Administration of the Slovakia.

Experimental animals

40 male Sprague Dawley (SD) rats aged 10 to 12 weeks and weighing from 250 to 500 grams were included in the experiment (Laboratory of research biomodels at Faculty of Medicine of the University of PJ Safarik).

Laboratory rats were randomly divided into five groups with equal number of rats:

1. healthy rats – control group,
2. rats after 2/3 liver resection without application of hepatocytes,
3. rats after TAA (thioacetamide) liver damage without application of hepatocytes,
4. rats after 2/3 liver resection with hepatocyte transplantation,
5. rats after TAA liver damage with hepatocyte transplantation.

In the observed groups of rats (group 2 and 4), we performed 2/3 liver resection according to Higgins and Anderson. Liver damage in group 3 and 5 was achieved by intraperitoneal administration of TAA at a dose of 175 mg/kg. Before surgery, the rats were

[1]Ist Department of Surgery, PJ Safarik University, Faculty of Medicine and L Pasteur University Hospital, Kosice, Slovakia, [2]Department of Forensic Medicine, PJ Safarik University, Faculty of Medicine and L Pasteur University Hospital, Kosice, Slovakia, [3]IInd Department of Internal Medicine, PJ Safarik University, Faculty of MEDICINE and L Pasteur University Hospital, Kosice, Slovakia, and [4]Transplant Department, L Pasteur University Hospital, Kosice, Slovakia

Address for correspondence: Rastislav KALANIN, MD, PhD, Ist Department of Surgery, PJ Safarik University, Faculty of Medicine and L Pasteur University Hospital, Trieda SNP 1, SK-040 01 Kosice, Slovakia.

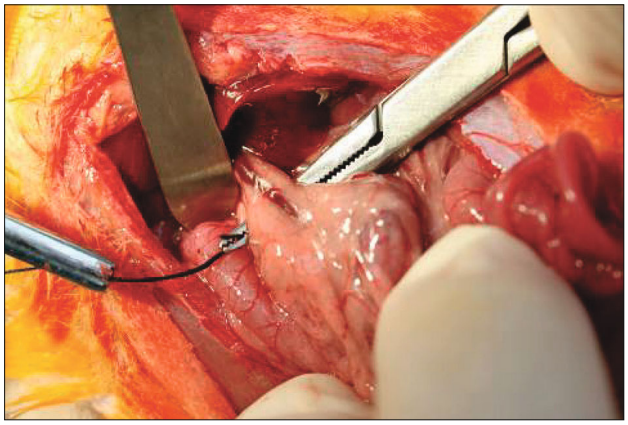

Fig. 1. Ligation of the portal vein before flushing. Source: author´s archive.

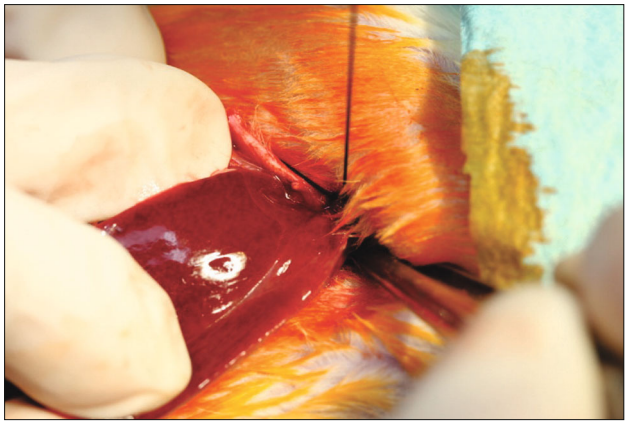

Fig. 2. Resection of liver lobe. Source: author´s archive.

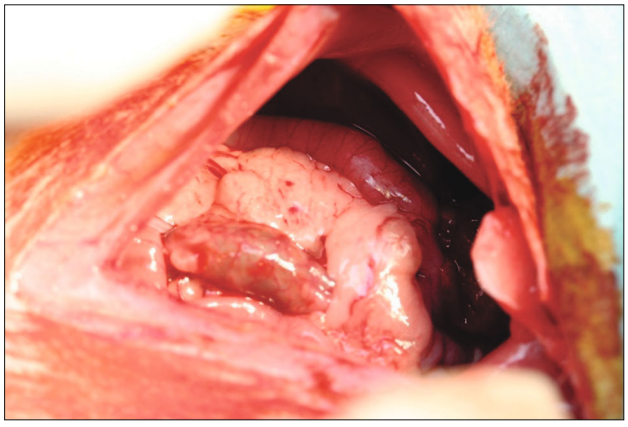

Fig. 3. Abdominal cavity after 2/3 liver resection. Source: author´s archive.

anesthetized using 15 mg/kg intraperitoneal dose of thiopental. After surgery, intramuscular analgesics were administered daily at a dose of 5 mg/kg of tramadol hydrochloride. After application of hepatocyte cells, experimental animals were given daily immunosuppressive treatment with Mycophenolate Mofetil (Cellcept 23 mg/kg).

Evaluation of liver function

In all groups, we monitored laboratory parameters (ALT, albumin, bilirubin) in the blood serum and weight changes during the experiment. We measured the laboratory values at five time points: at the beginning of the experiment, on day 1, 7, 14 and 21. Experimental animals were placed individually in cages with free access to water and food. The rats were weaned from food for 12 hours before surgery. We performed all procedures under sterile conditions.

Preparation of hepatocytes

Biological material – liver was obtained from healthy male Sprague Dawley rats. Laboratory rats were weaned from food 12 hours before surgery. The animals were put to sleep by administering intramuscular thiopental at a dose of 15 mg/kg.

After opening the abdominal cavity and visualizing the vascular structures, we ligated the portal vein and subsequently placed a cannula into the vein. The cannula was used to flush the liver with approximately 100 mL of saline solution at a temperature of 4 °C. After sufficient flushing, we removed the liver (Fig. 1).

The whole liver was washed 3 times in PBS (phosphate buffer saline) and cut into 1x1 cm pieces in a Petri dish with collagenase solution (5 mL). It was then transferred to 10 mL of stock collagenase solution and vortexed.

Subsequently, the tissue was incubated for 40 minutes, at 37 °C, in atmosphere of 5% CO_2 and 92% N_2. After the hold time, the whole tissue was filtered through filters (100 μm, 70 and 40 μm). Then it was centrifuged at 400 g, for 10 minutes and 4 °C. After centrifugation, about 3 mL of culture medium was added to the tissue. The culture medium contained: 10 % FBS (fetal bovine serum – Life technologies), penicillin (100 U/mL), (Gibco, Life Technologies), streptomycin (100 mg/mL) (Gibco, Life Technologies) and Amphotericin B (250 ng/mL), (Gibco, Life Technologies) and RPMI media (Gibco). After vortexing, the material was centrifuged again at 400 g, for 10 minutes and 4 °C. Subsequently, 500 μL of Diluent C (Sigma) and 500 μL of PKH26 dye (Sigma) were added to the cell pellet. The pellet was incubated for 3 minutes, and after incubation, 120 μL FBS was added and the pellet was incubated for 1 minute. Afterwards, 1 mL of culture medium was added, and hepatocytes were centrifuged at 300 g, for 10 minutes, washed with PBS, centrifuged again at 400 g, for 6 minutes, and washed with DMEM medium (Dulbecco's Modified Eagle Medium), (Gibco, Life technologies). Finally, DMEM medium was added to the cell pellet and this pellet was divided into Eppendorf tubes, 500 μL each (one Eppendorf tube contained $1x10^6$ cells). Subsequently, primary hepatocytes were applied to the rats with diseased part of the liver (1, 2).

Surgical performance of liver resection

After disinfecting the operating field, we opened the abdominal cavity. Subsequently, we tied off three lobes (Figs 2 and 3). After tying off the three lobes, we proceeded to perform 2/3 resection of the liver (3). The operation had to be performed with minimal blood loss. We performed the suturing of the abdominal cavity in anatomical layers.

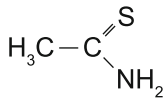

Fig. 4. Thioacetamide.

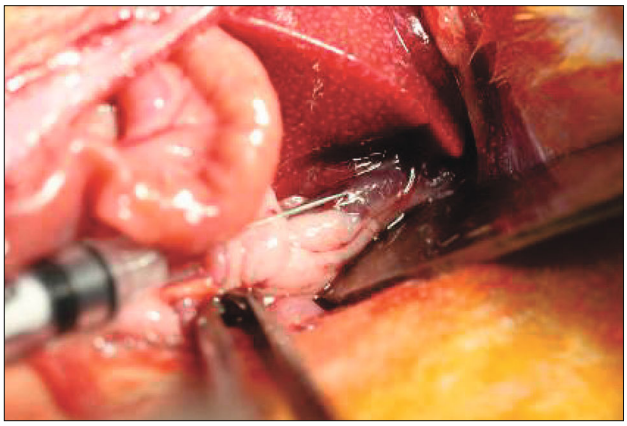

Fig. 5. Transplantation of hepatocytes into portal vein. Source: author´s archive.

Achieving liver damage with TAA

Thioacetamide (TAA) is one of hepatotoxins causing liver damage. A single dose of TAA can induce necrosis, repeated administration can lead to cirrhosis and liver cancer (4) (Fig. 4).

After the application of TAA, there was a significant increase in serum activity of ALT, AST and ALP, which is a consequence of damage to liver cells (5).

Before administration, the crystalline TAA had to be dissolved in saline solution. We administered the prepared TAA solution to experimental animals at intraperitoneal dose of 175 mg/kg. In animals, we observed changes of laboratory parameters in blood serum as well as weight changes.

Transplantation of the prepared hepatocytes

Same as with liver removal, laboratory rats were weaned from food 12 hours before surgery. The animals received intramuscular thiopental at a dose of 15 mg/kg.

After opening the abdominal cavity, we isolated the portal vein, which was required for administration of the hepatocytes (6). We administered 0.5 mL of a suspension of previously prepared hepatocyte cells into the portal vein, using an insulin syringe (Fig. 5).

Injection into the portal vein had to be performed carefully, as massive bleeding could occur with repeated injections (7). We transplanted hepatocytes within 4 hours of their preparation to ensure their survival. During this time, the hepatocytes were stored at the temperature of 4 °C.

Harvesting of the livers under observation

The duration of the experiment was 21 days, during which we damaged the liver in the observed groups of experimental animals by resection or by administration of TAA. We transplanted hepatocytes to selected groups and we monitored specific blood serum parameters and weight of all laboratory animals. The last step was the removal of the liver on day 21 of the experiment, which was then sent for histopathological analysis.

Preparation of slides for histopathological analysis

Before transplantation, we marked the hepatocytes with a fluorescent dye so that the transplanted hepatocytes could be detected using electron microscope at the end of the experiment.

After removal of the liver, we divided each slide in two samples. Before observing the slides, the collected tissues were processed and stained with hematoxylin/eosin for observation with a light microscope and frozen for observation with an electron microscope.

Staining of slides with hematoxylin/eosin

The harvested liver tissue samples were fixed for 48 hours in 10 % formaldehyde solution at room temperature. The samples were processed using standard histological technique, embedded in paraffin. Slices 3–4 μm thick were cut from the prepared paraffin blocks using a microtome, after deparaffinization the sections were stained with HE. The slides were analyzed using NIKON H550S Eclipse Ci light microscope with a MOTICAM 5 camera.

Preparation of slides for the electron microscope

The second group of slides observed with the electron microscope had to be frozen immediately after removal of the liver and observed for fluorescent staining, which can only be observed for a short time. After this time, the staining was no longer visible. For this reason, photographic records of the slides were made during observation, where the transplanted hepatocytes marked with a fluorescent dye were clearly visible.

Statistical methods

MS Excel 2021 and ArcusQuickstatBiomedical 1.1 software were used for statistical processing and data analysis. All monitored parameters were analyzed statistically - (ALT, bilirubin, albumin and weight). For individual parameters, we calculated the mean, number, standard deviation (SD), standard error of the mean (SEM), minimum, maximum, median, quartiles, percentage (%).

To express the significance of differences in the mean values of selected indicators between individual groups, we used unpaired t-test of for parametric data and non-parametric Mann-Whitney test. To evaluate the differences between the means of individual groups, one-way variance analysis, ANOVA variance analysis, or two-way analysis of variance, one-way analysis of variance for repeated measures, and the Newman–Keuls test. For all tests, we chose p=0.05 as the level of statistical significance.

Tab. 1. Normal values from the blood serum of a healthy rat.

	Normal values
Albumin	38–48 g/L
Bilirubin	3.42–9.4 mmol/L
ALT	0.29–0.51 μkat/L

Tab. 2. Value of ALT in the healthy control group, in the control group with liver damaged by resection, in the control group with liver damaged by TAA, in the group with liver resection and HC, in the group with liver damaged by TAA and HC.

	Healthy	Liver resection	Liver damaged by TAA	Liver resection +HC	Liver damaged by TAA + HC
Day 0	0.76	0.66	0.76	0.79	0.72
Day 1	0.65	12.91	16.89	12.89	21.57
Day 7	0.57	13.16	16.55	8.46	10.45
Day 14	0.53	13.19	20.08	1.87	4.76
Day 21	0.71	11.55	16.98	0.87	0.95

Tab. 3. Value of Bilirubin in a healthy control group, in the control group with liver damaged by resection, in the control group with liver damaged by TAA, in the group with liver resection and HC, in the group with liver damaged by TAA and HC.

	Healthy	Liver resection	Liver damaged by TAA	Liver resection + HC	Liver damaged by TAA + HC
Day 0	4.06	4.43	4.61	4.96	6.2
Day 1	4.38	12.01	12.85	12.12	13.98
Day 7	2.25	10.21	11.02	6.87	6.86
Day 14	3.08	10.96	12.33	5.35	4.75
Day 21	4.21	11.77	10.82	2.75	3.8

Results

The aim of the work was to monitor the possibility of cell therapy in rats after liver damage. In one group of animals, the liver was damaged by extensive 2/3 resection, in another group of animals, the liver was damaged by intraperitoneal administration of thioacetamide (TAA) at a dose of 175 mg/kg. After experimentally induced liver damage, isolated fresh rat hepatocytes (HC) at a dose of 10^9/mL were applied into the portal vein on the second day of treatment. There were 5 groups of animals observed in the experiment, group 1 with healthy rats with undamaged liver, the control group. Group 2: animals with liver damaged by 2/3 resection without treatment, group 3: animals with liver damaged by TAA without treatment, Group 4: animals with liver damaged by 2/3 resection, treated with HC; group 5: animals with liver damaged by TAA, treated with HC.

During surgery, the control groups of animals with liver damaged by 2/3 resection or by TAA application received 1 mL of saline solution into the portal vein. All animals were fasted for 12 hours before surgery, thiopental (15 mg/kg) was used for anesthesia during surgery. All procedures were carried out under aseptic conditions. Postoperatively, Tramadol Hydrochloride analgesic was administered for three days. During HC treatment, Mycophenolate Mofetil (Cellcept 23 mg/kg) was administered postoperatively (8).

Serum albumin (ALB), bilirubin (BIL), alanine transaminase (ALT) and animal body weight were monitored during the experiment (Tab. 1).

The blood sample was collected from the lateral tail vein of the rats (9). Parameters were recorded before liver injury, on day 1 after injury (by 2/3 resection or intraperitoneal TAA administration), when intraportal cell therapy was administered (HC), and subsequently on days 7, 14, and 21. The experiment ended on day 21 after the application of cell therapy. All animals were euthanized by intraperitoneal thiopental and killed by decapitation (10). This was followed by harvesting of the liver, which was fixed in 10 % formaldehyde at room temperature for the next 3 days before further processing.

From day 7 after the application of cell therapy (HC), in all treated groups with damaged liver (2/3 resection or TAA), the values of BIL and ALT gradually decreased compared to the control groups with damaged liver without treatment. On the other

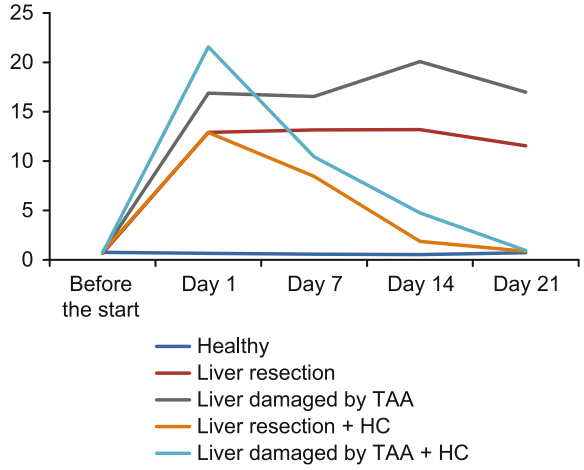

Fig. 6. Value of ALT in the healthy control group, in the control group with liver damaged by resection, in the control group with liver damaged by TAA, in the group with liver resection and HC, in the group with liver damaged by TAA and HC.

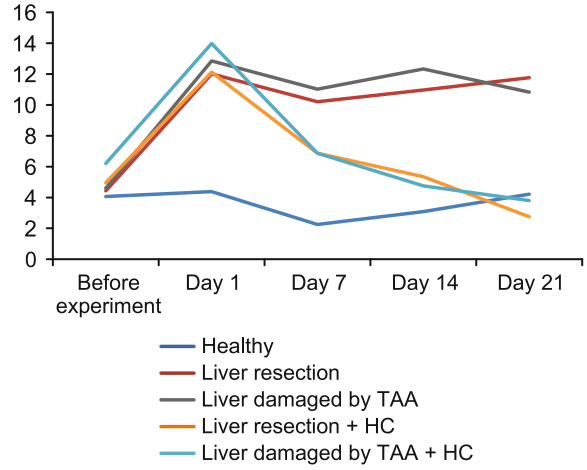

Fig. 7. Value of Bilirubin in a healthy control group, in the control group with liver damaged by resection, in the control group with liver damaged by TAA, in the group with liver resection and HC, in the group with liver damaged by TAA and HC.

Tab. 4. Value of Albumin in a healthy control group, in the control group with liver damaged by resection, in the control group with liver damaged by TAA, in the group with liver resection and HC, in the group with liver damaged by TAA and HC.

	Healthy	Liver resection	Liver damaged by TAA	Liver resection + HC	Liver damaged by TAA + HC
Day 0	26.78	29.53	27.93	30.88	35.01
Day 1	34.91	32.55	28.27	24.76	32.68
Day 7	34.21	30.9	28.15	28.33	31.48
Day 14	33.47	24.37	29.07	29.46	28.83
Day 21	30.79	20.34	23.16	29.73	30.55

Tab. 5. Weight in a healthy control group, in the control group with liver damaged by resection, in the control group with liver damaged by TAA, in the group with liver resection and HC, in the group with liver damaged by TAA and HC.

	Healthy	Liver resection	Liver damaged by TAA	Liver resection + HC	Liver damaged by TAA + HC
Day 0	453	447	461	470	430
Day 1	454	429	458	440	427
Day 7	467	418	463	442	435
Day 14	471	410	457	452	444
Day 21	479	401	453	461	453

hand, the values of ALB and weight of animals in treated groups increased.

The ALT values in the group 1 showed no statistically significant changes during the experiment, 0.76 μkat/L before the start of the experiment compared to 0.71 μkat/L at the end of the experiment on the day 21.

Values of ALT in group 2 showed statistically significant changes during the experiment – 0.66 μkat/L before the start of the experiment, followed by increase to 12.91 μkat/L after 2/3 liver resection, where liver damage was confirmed $p < 0.001$ and subsequently persistent high ALT values throughout the course of the experiment up to a value of 11.55 μkat/L on day 21 of the experiment, which, however, did not mean a statistically significant difference compared to the value after inducing liver damage.

Values of ALT in group 3 showed statistically significant changes during the experiment – 0.76 μkat/L before the start of the experiment followed by an increase to 16.89 μkat/L after TAA administration, where liver damage was confirmed with statistical significance of $p < 0.001$ and subsequently persistent high ALT values throughout the course of the experiment, at a value of 16.98 μkat/L on day 21 of the experiment, which, however, did not mean a statistically significant difference compared to the value after the induction of liver damage.

Values of ALT in group 4 showed statistically significant changes during the experiment – 0.79 μkat/L before the start of the experiment, an increase to 12.89 μkat/L after 2/3 liver resection, where liver damage was confirmed $p < 0.001$ and a subsequent decrease to 0.87 μkat /L on day 21 of the experiment, confirmed by $p < 0.001$.

Values of ALT in group 5 showed statistically significant changes during the experiment – 0.72 μkat/L before the start of the experiment rising to 21.57 μkat/L after TAA application, where liver damage was confirmed with $p < 0.001$ and then decreased to 0. 95 μkat/L on day 21 of the experiment, confirmed by statistical significance of $p < 0.001$ (Tab. 2, Fig. 6).

Similar significant results were found when observing the values of BIL. In group 1 at the beginning of the experiment 4.06 mmol/L compared to the value of 4.21 mmol/L at the end of the experiment, without statistical significance. Values of BIL group 2 showed statistically significant changes during the experiment – 4.43 mmol/L before the start of the experiment, followed by an increase to 12.01 mmol/L after 2/3 resection of the liver, $p < 0.001$, and subsequently persistently high BIL values during the

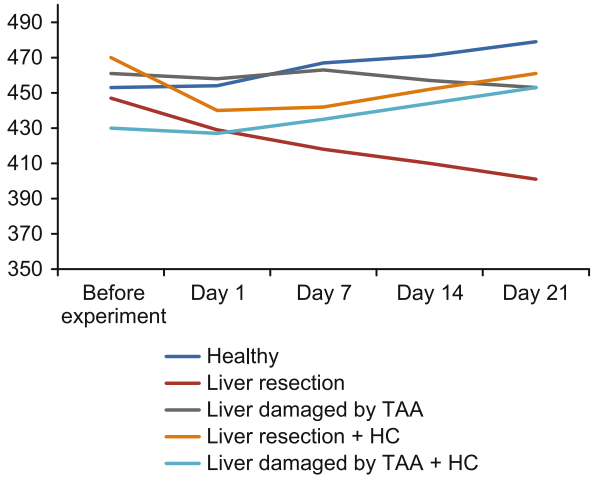

Fig. 8. Weight in the healthy control group, in the control group with liver damaged by resection, in the control group with liver damaged by TAA, in the group with liver resection and HC, in the group with liver damaged by TAA and HC.

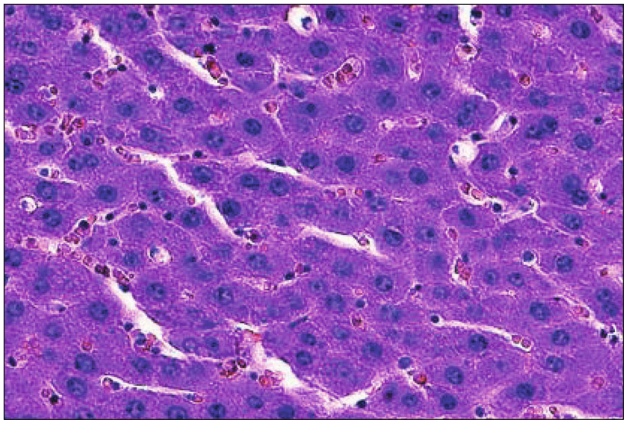

Fig. 9. Slide from undamaged liver, without HC transplantation. Source: author´s archive.

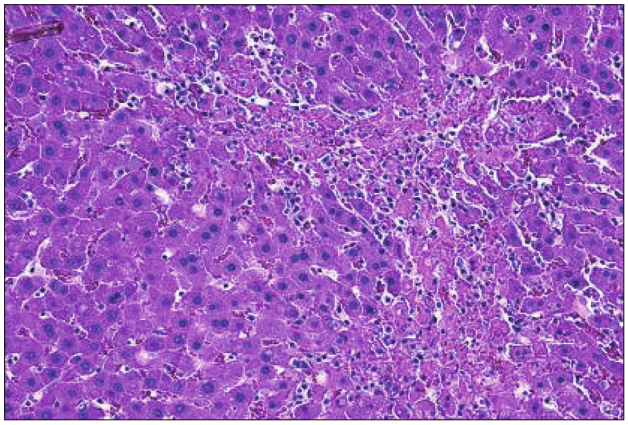

Fig. 10. Liver necrosis after administration of TAA. Source: author´s archive.

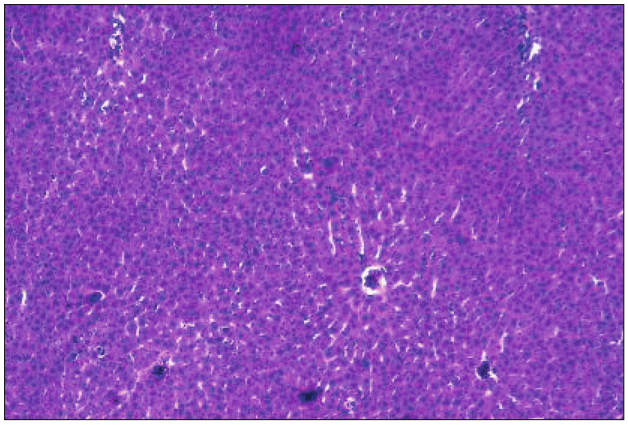

Fig. 11. Necrosis at the edges of resected liver. Source: author´s archive.

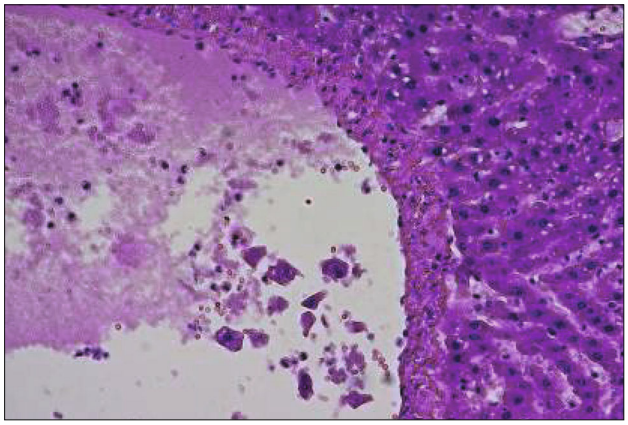

Fig. 12. Transplanted hepatocytes in liver damaged by TAA. Source: author´s archive

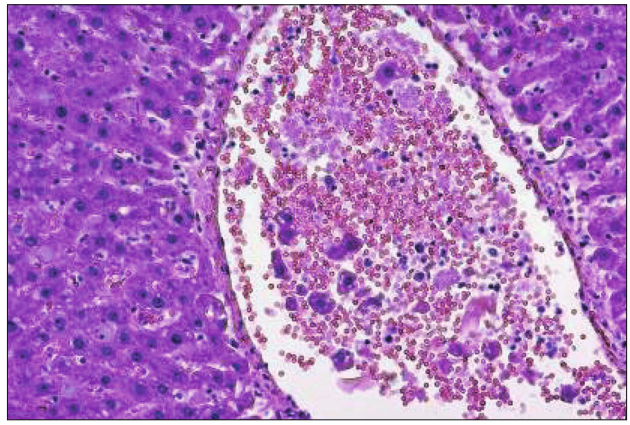

Fig. 13. Transplanted hepatocytes in the central vein. Source: author´s archive.

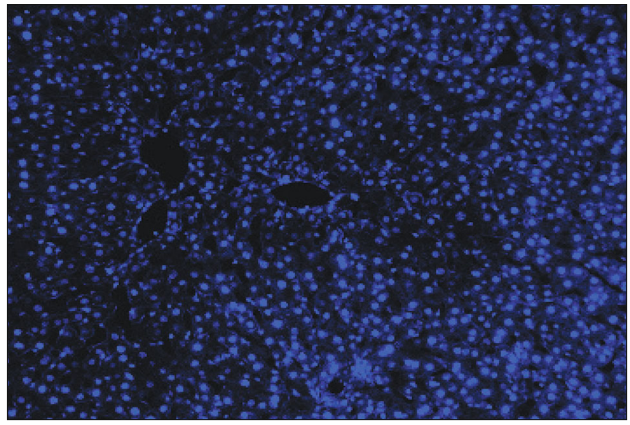

Fig. 14. Slide from undamaged liver, without HC transplantation. Source: author´s archive.

entire course of the experiment up to the value 11.77 mmol/L on day 21 of the experiment. Values of BIL in group 3 during the experiment – 4.61 mmol/L before the start of the experiment, then rising to 12.85 mmol/L after TAA administration, $p < 0.001$ and persisting throughout the entire course of the experiment, value of 10.82 mmol/L on day 21 of the experiment.

In group 4, the values were 4.96 mmol/L before the start of the experiment, increased to 12.12 mmol/L after 2/3 resection of the liver, $p < 0.001$ and then decreased to 2.75 mmol/L on day 21 of the experiment, $p < 0.001$. In group 5, the values were 6.20 mmol/L at the beginning of the experiment, followed by an increase to 13.98 mmol/L after TAA application, $p < 0.001$ and a decrease to 3.8 mmol/L on day 21 of the experiment, $p < 0.001$ (Tab. 3, Fig. 7).

Similar significant results were found when observing the values of ALB. Group 1 showed no statistical significance (26.78 g/L and 30.79g/L at beginning of the experiment and at the end of the experiment respectively). ALB values in group 2 (beginning 29.53 g/L, decrease at the end 20.34 g/L), $p < 0.01$. ALB in group 3 – 27.93 g/L decreased to 23.16 g/L, $p < 0.01$ on day 21 of the experiment.

In group 4 (beginning 30.88g/L, damaged liver 24.76 g/L, end of experiment 29.73g/L), $p < 0.01$. In group 5 (beginning 35.01 g/L, damaged liver 32.68 g/L, after treatment 30.55 g/L), $p < 0.01$ (Tab. 4).

When monitoring weight, an increase was detected in groups 1, 4 and 5, but in the control group with liver damage and 2/3 resection and TAA, there was a decrease, $p < 0.001$ (Tab. 5, Fig. 8).

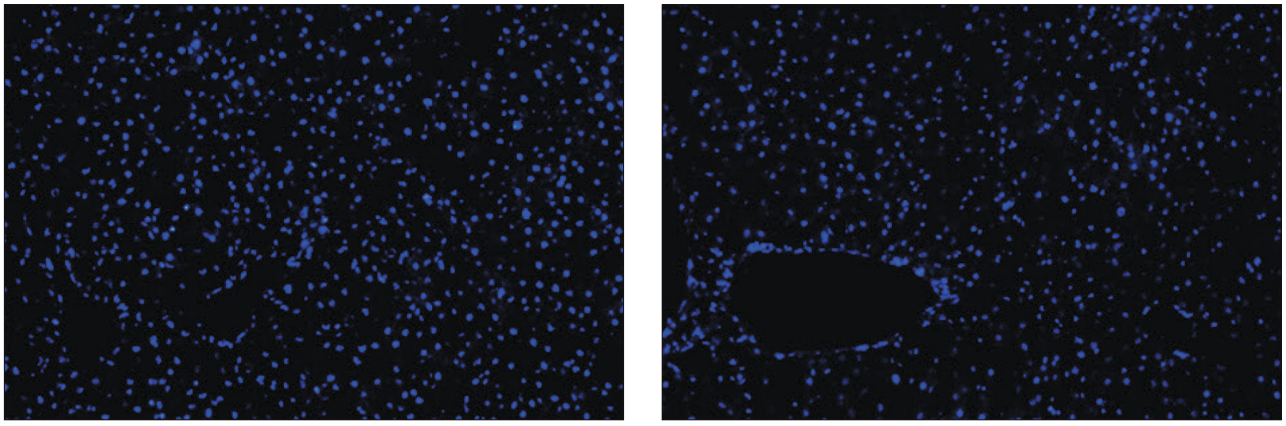

Fig. 15. Liver damaged by resection and administration of TAA without HC transplantation. Source: author´s archive.

Histological analysis of rat liver slides

Histopathological and microscopic image analysis was used to evaluate the slides obtained from the removed rat livers, which demonstrated the survival of the transplanted hepatocytes at the end of the experiment. Two methods of evaluation were used - with a light and electron microscope.

Observation of slides with a light microscope

Preparations from individuals without liver damage and without HC application did not show any variations from the norm (Fig. 9). Healthy hepatocytes are visible.

When the hepatotoxic substance TAA was administered intraperitoneally to healthy rats, we observed necrosis of the entire liver tissue visible in Figure 10.

The slides from resected livers without HC transplantation showed the presence of necrotic tissue at the resection edge only, as shown in Figure 11.

Figures 12 and 13 show slides of liver tissue damaged by resection and administration of TAA. Both images show the central vein where the presence of transplanted hepatocytes can be observed.

Electron microscope

In slides of liver tissue observed under the electron microscope, we found the presence of transplanted hepatocytes stained red due to the staining of hepatocytes by fluorescence labeling before the transplantation itself. Figures 14 and 15 show slides in groups 1, 2 and 3 in which the prepared hepatocytes were not transplanted. The detected stained transplanted hepatocytes were seen in Figures 16 and 17 in groups 4 and 5, which were transplanted with hepatocytes after liver damage.

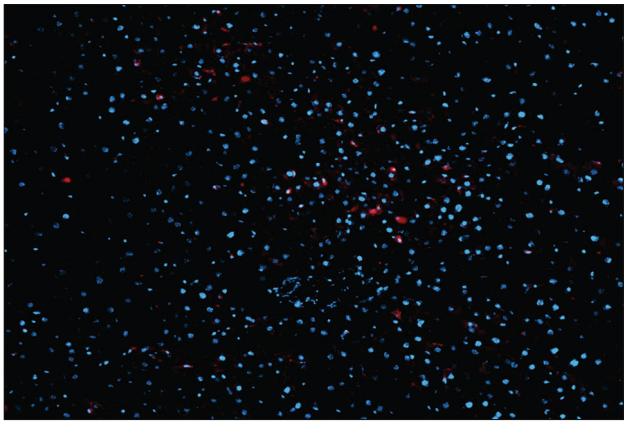

Fig. 16. Liver damaged by resection after HC transplantation. Source: author´s archive.

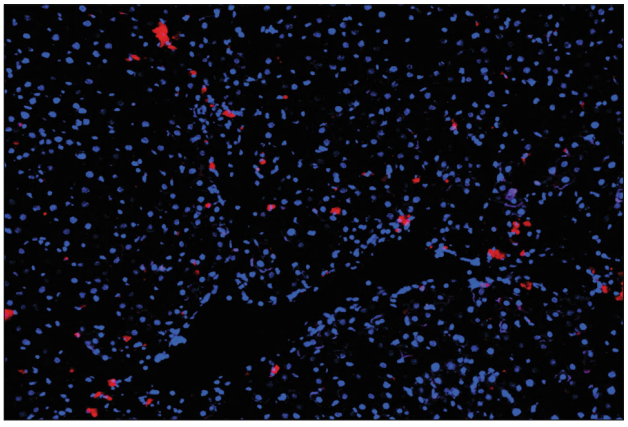

Fig. 17. Liver damaged by TAA after HC transplantation. Source: author´s archive.

Discussion

In our experiment, we observed the effect of cell therapy in rats, with livers damaged in two ways. In the first group, we damaged the liver by extensive 2/3 resection of the liver, and in the second group of individuals, we damaged the liver by intraperitoneal administration of thioacetamide. After the liver was damaged by the methods described, we transplanted freshly isolated hepatocytes by injection into the portal vein of selected groups of experimental animals (group 4 and 5).

Control groups of animals after 2/3 resection of the liver, or after the application of TAA were injected during operation in

the same way with saline solution of the same volume into the portal vein as a placebo. Individuals who underwent HC transplantation were given daily immunosuppressive therapy in the postoperative period.

During the entire course of the experiment, we monitored the serum values of ALB, BIL, ALT and the weight of the experimental animals in all individuals at the same time point. We ended the experiment on day 21. In the next step, the liver was harvested for the purpose of histological evaluation of tissue samples.

Regarding the results, we observed a gradual decrease in BIL and ALT in individuals who were transplanted with HC already from day 7 of the experiment – when compared with the control groups with damaged liver without treatment, and an increase in the values of ALB and body weight in the treated groups.

After the end of the experiment, we evaluated the histological preparations of all individuals, which were made from the livers of individual rats in individual groups. In the control group of animals with undamaged liver, who did not receive cell therapy, no deviations from the histological norm were noted.

In the group with liver damaged by administration of TAA and without cell therapy, we observed necrosis of the liver parenchyma in the histological image.

In the histological images of individuals after 2/3 liver resections without hepatocyte transplantation, necrotic changes were noted at the liver resection line, the rest of the liver tissue was intact.

In the groups of animals in which we damaged 2/3 of the liver by resection or by administration of TAA and subsequently transplanted with HC, the transplanted HC (hepatocytes) were clearly identified during the histological analysis, as we marked them with a fluorescent dye in the preparation step before the actual transplantation in order to identify them and prove their presence in the particular tissues.

From the results described above, it follows that in our experiment we clearly managed to prove the therapeutic effect of hepatocyte transplantation in individuals after 2/3 liver resections and also after TAA liver damage – through biochemical and histological analysis of samples. The results of our experiment represent a very promising basis for the possible future clinical use of this therapeutic procedure in human medical practice.

However, the selection of the optimal location for hepatocyte transplantation with regard to their survival and storage of hepatocytes after harvesting remains a fundamental challenge (11). In the future, it will be necessary to address the best procedure for hepatocyte cryopreservation to ensure their availability when needed by the patient, their sufficient viability after thawing, and long-term longevity and stability after transplantation needs to be resolved. An important step in the field of cell transplantation could be the resolution to the problem of the body's immunological reaction to transplanted cells. At present, the encapsulation method that does not require immunosuppressive treatment, appears promising.

Considering the fact that the studies published so far, focusing on hepatocyte transplantation, demonstrate a relatively positive effect of this treatment method, it can be assumed that hepatocyte transplantation will bring the desired treatment effect to patients after extensive liver resections due to cancer, severe liver failure (bridging to liver transplantation), chronic viral hepatitis, liver cirrhosis and chronic liver failure, liver graft rejection. Additional use for this therapy may also be possible in patients with hereditary liver conditions (12).

The available publications describes several options for selecting the target site for hepatocyte application. Most often, hepatocytes are administered either to the liver or to the spleen. Other potential target sites for transplantation reported in the literature are the peritoneal cavity, pancreas, skeletal muscle, salivary glands, thyroid gland and, subcutaneous tissue, kidneys or fatty tissue around the scapula (13). Hepatocytes transplanted to these places have different levels of survival and functionality and provoke an immune response of the host organism. This must be prevented by immunosuppressive treatment.

From the point of view of survival of transplanted hepatocytes, the best site for administration is the liver itself. Administration of hepatocytes to the cirrhotic liver through the portal vein is described as the most favorable method of application. However, in the case of individuals with liver cirrhosis, a large proportion of hepatocytes passes through the liver or via portocaval junctions to other tissues, mainly the lungs, where they are destroyed, which is associated with the risk of pulmonary embolism. Because of this fact, it is absolutely necessary to exclude individuals with advanced liver cirrhosis before transplantation of hepatocytes through the portal vein. When hepatocytes are administered to the portal vein, portal hypertension occurs for a few hours, but it resolves over time (14). In hepatocyte transplantation, there is a generally accepted rule the amount of hepatocytes that can be administered at one time should not exceed the equivalent of 10 % of the liver tissue. However, this procedure can be carried out repeatedly (13). When hepatocytes are transplanted into the peritoneal cavity, necrosis of these cells occurs very quickly (15). The hepatocyte survival rate can be increased by protecting them with a biocompatible membrane, the so-called encapsulation. The benefit of encapsulation is that the administration of these hepatocytes does not cause an immune reaction and does not require the administration of immunosuppressants (16).

Considering the above facts, when planning our experiment, we decided to transplant hepatocytes into the liver parenchyma, whereas we applied the hepatocytes into the portal vein of the experimental animals.

References

1. Shen L, Hillebrand A, Wang DQ, Liu M. Isolation and primary culture of rat hepatic cells. J Vis Exp 2012; (64): 3917. DOI: 10.3791/3917.

2. Charni-Natan M, Goldstein I. Protocol for Primary Mouse Hepatocyte Isolation. STAR Protoc 2020; 1 (2): 100086. DOI: 10.1016/j.xpro.2020.100086.

3. Kamali C, Kamali K, Brunnbauer P, Splith K, Pratschke J, Schmelzle M, Krenzien F. Extended liver resection in mice: state of the art and pitfalls-a systematic review. Eur J Med Res 2021; 26 (1): 6. DOI: 10.1186/s40001-020-00478-3.

4. Wallace MC, Hamesch K, Lunova M, Kim Y, Weiskirchen R, Strnad P, Friedman SL. Standard operating procedures in experimental liver research: thioacetamide model in mice and rats. Lab Anim 2015; 49 (Suppl 1): 21–29. DOI: 10.1177/0023677215573040.

5. Zargar S, Wani TA, Alamro AA, Ganaie MA. Amelioration of thioacetamide-induced liver toxicity in Wistar rats by rutin. Int J Immunopathol Pharmacol 2017; 30(3): 207–214. DOI: 10.1177/0394632017714175.

6. Ogasawara H, Inagaki A, Fathi I, Imura T, Yamana H, Saitoh Y, Matsumura M, Fukuoka K, Miyagi S, Nakamura Y, Ohashi K, Unno M, Kamei T, Goto M. Preferable Transplant Site for Hepatocyte Transplantation in a Rat Model. Cell Transplant 2021; 30: 9636897211040012. DOI: 10.1177/09636897211040012.

7. Heath RD, Ertem F, Romana BS, Ibdah JA, Tahan V. Hepatocyte transplantation: Consider infusion before incision. World J Transplant 2017; 7 (6): 317–323. DOI: 10.5500/wjt.v7.i6.317.

8. Loukopoulos I, Sfiniadakis I, Pillai A, Konstantoulakis M, Androulakis G, Bonatsos V, Zografos G, Papalois A. Mycophenolate mofetil and sirolimus in hepatocyte transplantation in an experimental model of toxic acute liver failure. J Invest Surg 2014; 27 (4): 205–213. DOI: 10.3109/08941939.2013.879967.

9. Lee G, Goosens KA. Sampling blood from the lateral tail vein of the rat. J Vis Exp 2015; (99): e52766. DOI: 10.3791/52766.

10. Laferriere CA, Pang DS. Review of Intraperitoneal Injection of Sodium Pentobarbital as a Method of Euthanasia in Laboratory Rodents. J Am Assoc Lab Anim Sci 2020; 59(3): 254–263. DOI: 10.30802/AALAS-JAALAS-19-000081.

11. Mazari-Arrighi E, Okitsu T, Teramae H, Aoyagi H, Kiyosawa M, Yano M, Chatelain F, Fuchs A, Takeuchi S. In vitro proliferation and long-term preservation of functional primary rat hepatocytes in cell fibers. Sci Rep 2022; 12(1): 8813. DOI: 10.1038/s41598-022-12679-3.

12. Iansante V, Mitry RR, Filippi C, Fitzpatrick E, Dhawan A. Human hepatocyte transplantation for liver disease: current status and future perspectives. Pediatr Res 2018; 83 (1–2): 232–240. DOI: 10.1038/pr.2017.284.

13. Koblihová E.: Transplantace hepatocytů – současný stav. Bulletin HPB 2005; 13 (3 – 4).

14. Fox IJ. Hepatocyte Transplantation. Gastroenterol Hepatol (N Y) 2014; 10 (9): 594–596.

15. Habibullah CM, Syed IH, Qamar A, Taher-Uz Z. Human fetal hepatocyte transplantation in patients with fulminant hepatic failure. Transplantation 1994; 58 (8): 951–952. DOI: 10.1097/00007890-199410270-00016.

16. Dhawan A. Clinical human hepatocyte transplantation: Current status and challenges. Liver Transpl 2015; 21 (Suppl 1): S39–44. DOI: 10.1002/lt.24226.

Received July 6, 2023.
Accepted August 30, 2023.