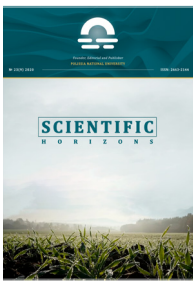
SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua

Scientific Horizons, 24(5), 9-18



UDC 619:616.36
DOI: 10.48077/scihor.24(5).2021.9-18

Stimulation of the Liver Regeneration with Bone Marrow Mesenchymal Stem Cells

Martina Vidová Uğurbaş[1,2*], Jana Kaťuchová[1,2], Darina Petrášová[1], Timea Špaková[1], Jozef Radoňak[1,2]

[1]Pavol Jozef Šafárik University in Košice
040 11, Trieda SNP 1, Košice, Slovak Republic

[2]University Hospital of L. Pasteur in Košice
040 11, Trieda SNP 1, Košice, Slovak Republic

Article's History:
Received: 20.07.2021
Revised: 25.08.2021
Accepted: 28.09.2021

Suggested Citation:
Vidová Uğurbaş, M., Kaťuchová, J., Petrášová, D., Špaková, T., & Radoňak, J. (2021). Stimulation of the liver regeneration with bone marrow mesenchymal stem cells. *Scientific Horizons*, 24(5), 9-18.

Abstract. The main objective of our experiment was to prove the effect of mesenchymal stem cells of bone marrow (MSCs) on the stimulation of liver regeneration. The attention has been paid to adaptation of stem cells to the new environment and their transfer to anatomical structures. The experiment included 40 male Sprague Dawley (SD) rats aged 10 to 12 weeks. Biomodels were divided into five groups in the same number (n=8). Group 1 consisted of a control sample of eight healthy rats. Group 2 consisted of eight rats after liver resection without application of MSCs. Group 3 was after liver resection and application of MSCs. Group 4 after liver injury induce with Thioacetamide (TAA), without transplantation of MSCs. Group 5 was after chemical damage to the liver by TAA administration and MSCs transplantation. The process of stimulation of the liver was observed based on the laboratory values of alanine aminotransferase (ALT), albumin and bilirubin. The weight of the rats in each group was also compared. Animals were sacrificed after 1 day, 7 days, 14 days, and 21 days. In our experiment we found a statistically significant decrease in ALT ($P \leq 0.001$) and bilirubin ($P \leq 0.001$) was observed in the groups 3 and 5 (treated with MSCs) compared to the groups without MSCs (Groups 2 and 4). The increase in the albumin levels in the groups 3 and 5 was statistically significant. The results of our experiment led us to the conclusion, the transplantation of MSCs has important effect for the treatment and stimulation of liver regeneration following injury. MSCs administration may be extremely useful in a number of clinical applications in the treatment of liver tumors. It will allow us to perform extensive resection of the liver without risk of liver failure

Keywords: stem cells, thioacetamid, liver resection, liver regeneration

*Corresponding author

INTRODUCTION

Recent research findings highlight the potential for the use of stem cells in the future [1]. Mesenchymal stem cells are multipotent cells able of self renewal and differentiation into several cell lines, including chondrocytes, osteoblasts and adipocytes. This type of stem cells isusually isolated from bone marrow, they can also be obtained from several neonatal and adult tissues, including umbilical cord, and fat tissue [2]. Under rare conditions, adult SCs can be reprogrammed and produce cell lines different from the original tissue. This phenomenon is called plasticity of stem cells. A change in the differentiation potential occurs when the stem cell leaves its original site and settles in the new tissue and then under the influence of new signal molecules, it can change the destiny of the stem cell so much that it starts producing different cell lines [3; 4]. All these properties of MSCs, together with the enormous ability to differentiate into specialized cells – in our case hepatocytes – make them the most suitable candidates for cell therapy in the area of liver regeneration [5; 6].

Primary liver cancer is the second leading cause of cancer related death worldwide and therefore a major public health challenge. Primary liver cancer includes hepatocellular carcinoma, intrahepatic cholangiocarcinoma, and other rare tumors, notably fibrolamellar carcinoma and hepatoblastoma [7]. The tumor size is one of the important predictive factors affecting treatment and early diagnosis is decisive for favorable prognosis [8].

In the liver tumors, the liver resection is the most effective treatment. Statistics show that curative resection is possible in about 47% of the patients with primary liver cancer, about 20% of the patients are inoperable before surgery, and 33% of the patients at surgery. The result of liver resections depends to a large extent on the functional capacity of the remaining liver residue. It is known that 20% of healthy liver is sufficient to maintain liver function after resection. In a cirrhotic liver, a minimum of 40% well-perfused liver-volume must remain *in situ* after resection. Liver resections in cirrhotic patients show a significantly increased mortality and morbidity, the functional capacity of the remaining liver residue is substantially reduced. Postoperative liver failure is the most serious and life-threatening complication after liver resection [9].

Existing experimental studies on mice by M. Kaibori et al. [10], "attempted to stimulate liver regeneration by stem cell transplantation (SCs) taken from bone marrow of mice. After isolation of SCs, they were subsequently transplanted by injection into two experimental groups. In the first experimental group the MSCs were transplanted by injection into the portal vein. In the second experimental group the MSCs were transplanted by injection into the tail vein, and in the third control group physiological solution was injected into the portal vein" [10] performed 70% of hepatectomy, which was described by Higgins and Anderson [11]. The mice were divided into three groups as listed and individual solutions were injected into the liver immediately after hepatectomy. In the experiment, they focused on assessing the liver regeneration according to the size, liver tissue histology, and statistical analysis of the results. Transplantation of MSCs has shown a great promise to improve the tissue regeneration in various acute and chronic diseases [10].

The main purpose of this study was to confirm and manifest the effect of bone marrow-derived mesenchymal stem cells on the stimulation of liver regeneration after injury.

MATERIALS AND METHODS

All the experimental conditions of the experiment were in compliance with the European rules of ethical standards of animal care. The experiment was approved by the Ethics committee of the Faculty of Medicine, University of Pavol Jozef Šafárik, and the State Veterinary and Food Administration of the Slovak Republic. Forty male Sprague Dawley (SD) rats at the age of ten to twelve weeks with an initial weight of 250 to 350 grams were included. The biomodels were randomly divided into five groups in the same number (n=8):

Group 1: Healthy rats were a control group.

Group 2: Rats after radical resection of the liver without application of MSCs.

Group 3: Rats after radical resection of the liver with application of MSCs.

Group 4: Rat after liver damage with TAA (thioacetamide) induction without application of stem cells.

Group 5: Rat after liver damage with TAA induction with application of stem cells into the liver.

Mechanical damage of the liver – liver resection. At liver resections in experimental rats we proceeded taken into account the individual lobes of the liver. The experimental animals used in the study began to starve 12 hours before surgery, in the morning we took away water. The animals were anaesthetized by intramuscular administration of Thiopental at a dose of 15 mg/kg, after which the experimental animals were placed in the incubator for 10-15 min. Once the required depth of anaesthesia had been reached, we started operating. After placing the experimental animal on the operating table, we prepared an operating field. We performed a laparotomy. After opening the abdominal cavity, we performed radical resection of the liver by ligating the individual lobes of the liver and then resecting them (Fig. 1, 2). Hepatic resections in the rats of the groups observed were performed at the range of 70% as described Higgins and Anderson [11]. Hepatic impairment by TAA (Thio-acetamide). TAA is a chemical used to make catalysts, stabilizers, polymerization inhibitors, galvanic cells, pesticides, and dyes. It seriously damages the liver [12].

Figure 1. *Ligation of the liver lobe – hemihepatectomy*

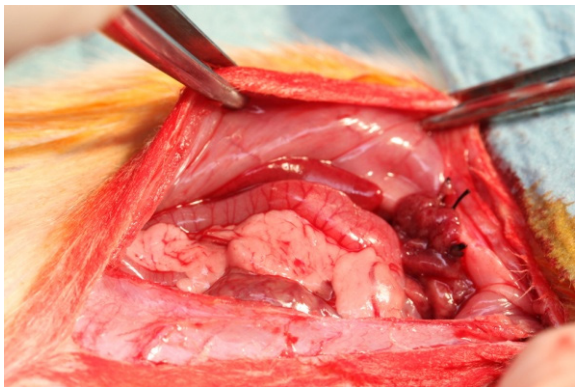


Figure 2. *Condition after liver resection*

Twenty-four hours before the intraperitoneal administration of TAA, we took blood from the tail vein approximately 2 ml for laboratory testing of ALT, bilirubin, and albumin. Subsequent samplings were performed 24 hours after TAA administration on days 1, 7, 14, and 21. The hepatic damage was induced by intraperitoneal TAA at the dose of 175 mg/kg [13]. The experimental animals were separated and labelled. The weight loss in a given group due to induced liver failure was continuously detected. Before the surgery, the animals were anesthetized using the intraperitoneal administration of thiopental. As an analgesic, tramadol hydrochloride (Grünenthal GmbH, Aachen, Germany) was administered intramuscularly at a dose of 5 mg/kg in the post-operative period.

All the animals in the study began to starve 12 hours before the stem cell application. As anaesthetic used thiopental 15 mg/kg. After preparation of the operating field, the longitudinal incision opened the abdominal cavity from the processus xiphoideus downwards. Suspension of mesen-chymal stem cells was given via an insulin syringe to the vena portae. The MSC suspension (1x106 in a volume of 1 ml of culture solution). In the experimental rats after previous liver resection, the stem cells were applied to the vena portae. In the experimental rats with chemical impairment of the liver by TAA, the stem cells were also transplanted into the vena portae (Fig. 3, 4). The abdominal cavity wall was closed in individual anatomical layers.

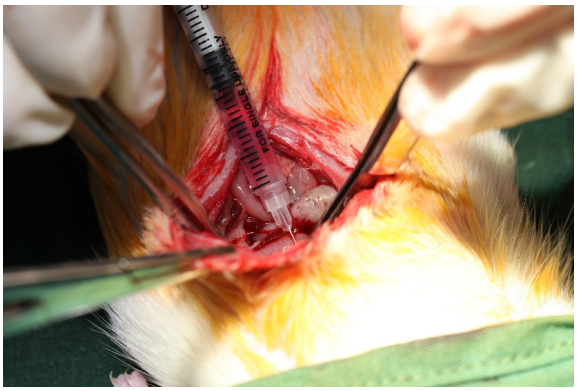


Figure 3. *Application of MSCs to the v. portae*

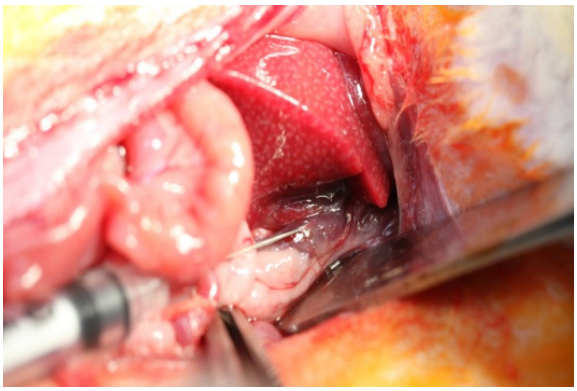


Figure 4. *Presented v. portae*

The experiment was continuously finished on day 21 after transplantation of the MSCs. The rats were anaesthetized with an increased dose of thiopental anaesthesia, and then the animals were sacrificed by decapitation. The liver collection itself was performed after the preparation of the field under aseptic conditions. In rats after the previous resection, only the remaining part was resected, which was 20-30%. In rats with chemical damage of the liver after TAA administration, hepatectomy was performed.

RESULTS AND DISCUSSION

The results were obtained by comparing the ALT, albumin and bilirubin values in the individual groups of experimental animals, typification of mesenchymal stem cells, histopathological and microscopic analysis.

Group 2 – the experimental rats with mechanically damaged liver after resection without MSCs transplantation – in this group an increase in the laboratory ALT values, bilirubin and, on the contrary, a decrease in the albumin values was observed;

Group 3 – the experimental rats with damaged liver after resection and with MSCs transplantation – in this group, an increase in the laboratory values of ALT, bilirubin immediately after resection was observed. In the following days, ALT and bilirubin decreased and albumin increased. This result demonstrates the therapeutic effect of transplanted mesenchymal cells and their positive effect on the liver regeneration.

Group 4 – the experimental rat with the liver chemically impaired with TAA without MSCs transplantation – in this group, likewise in the group 2 an increase

of laboratory values of ALT, bilirubin, and, on the contrary, decreases in the albumin values were observed. In the following days, the values did not alter as regards ALT and bilirubin. The albumin value did not increase.

Group 5 – the experimental rats with damaged liver by TAA application with MSCs transplantation – in this group, an increase in laboratory values of ALT, bilirubin was observed after resection. In the following days, a decrease in the ALT and bilirubin values, and an increase in albumin were observed. The graphical representation of our results is at Tables 1-4 and in Figures 5-12.

***Table 1**. The alanine transaminase (ALT) values obtained in the individual monitored groups*

	0 day	1st day	7th day	14th day	21st day
Group 1	0.76	0.65	0.57	0.54	0.71
Group 2	0.66	12.92	13.16	13.19	10.41
Group 3	0.59	12.5	7.92	3.43	2.21
Group 4	0.73	16.89	16.25	20.08	16.98
Group 5	0.69	20.85	14.44	4.69	1.48

***Table 2**. The albumin values obtained in the individual monitored groups*

	0 day	1st day	7th day	14th day	21st day
Group 1	26.78	34.91	34.21	33.47	30.79
Group 2	29.53	34.25	30.90	24.37	19.82
Group 3	28.48	34.22	29.43	26.76	22.73
Group 4	29.73	29.27	28.15	29.07	23.16
Group 5	39.25	27.23	32.65	29.12	29.99

***Table 3**. The bilirubin values obtained in the individual monitored groups*

	0 day	1st day	7th day	14th day	21st day
Group 1	4.06	4.38	2.25	3.08	4.21
Group 2	3.47	12.01	10.21	10.25	11.77
Group 3	4.43	12.22	5.33	5.73	4.01
Group 4	4.61	12.85	10.85	10.85	10.82
Group 5	5.07	14.12	6.96	4.95	3.2

***Table 4**. The weight values obtained in the individual monitored groups*

	0 day	1st day	7th day	14th day	21st day
Group 1	435.25	454.87	467.375	471.87	479.75
Group 2	447.75	429.5	418	410	401.37
Group 3	435.125	431.12	435	442	448.62
Group 4	430.62	452.62	463.12	457.5	454.12
Group 5	436.25	423.37	432.5	442.37	452.64

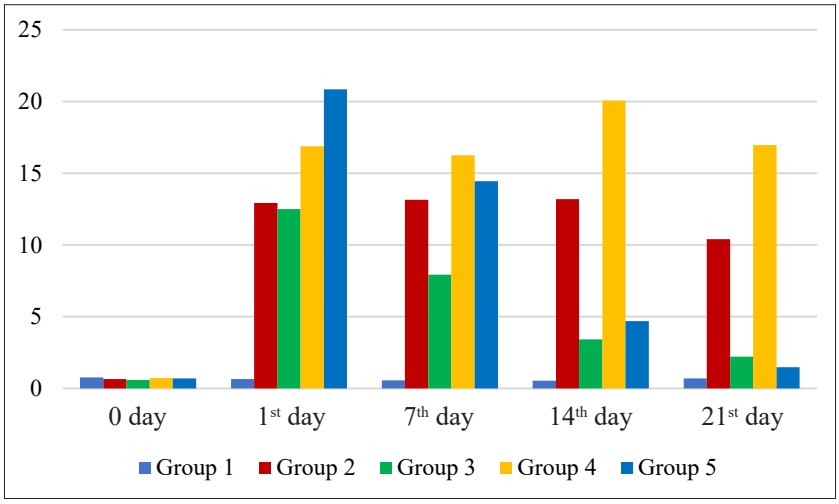


Figure 5. *Graphical evaluation of ALT*

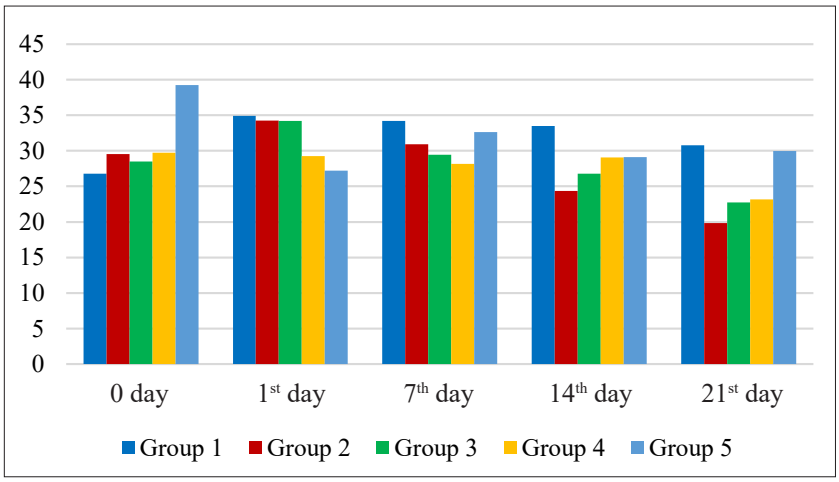


Figure 6. *Graphical evaluation of the mean values of albumine*

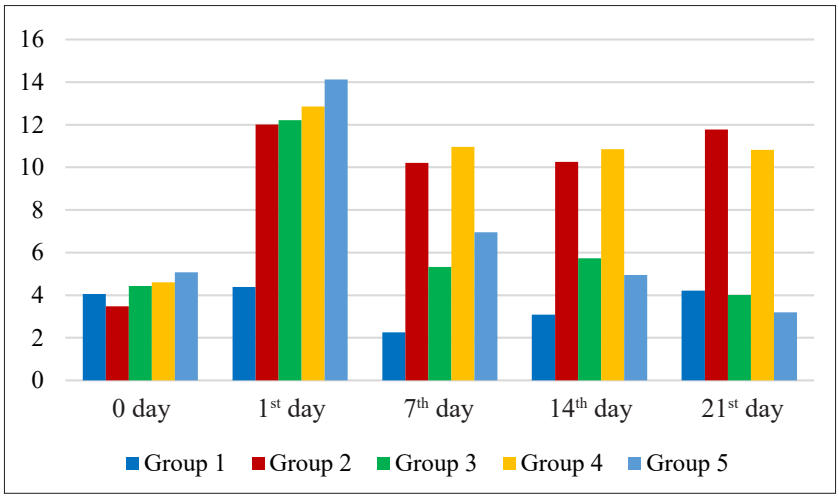


Figure 7. *Graphical evaluation of bilirubin*

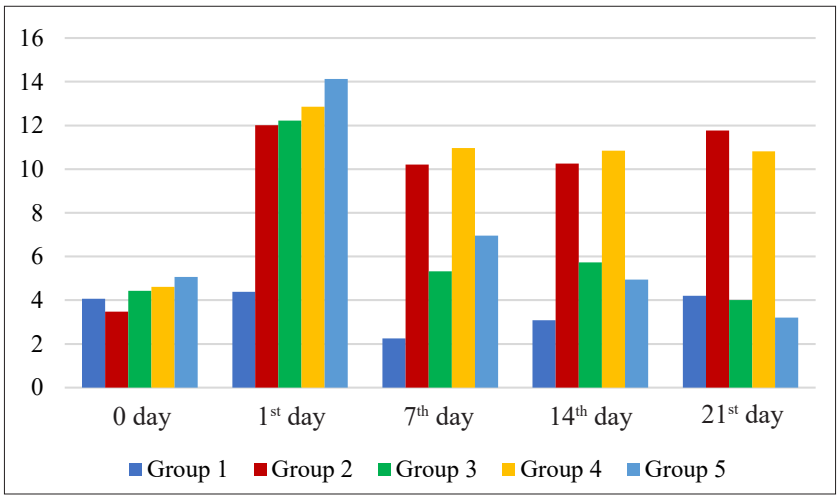


***Figure 8**. Weight dependence chart*

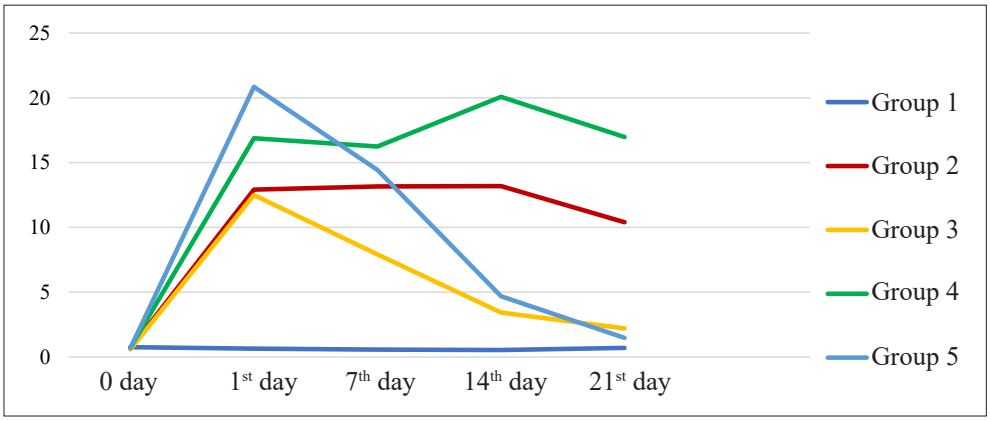


***Figure 9**. The graphic evaluation of the alanine transaminase (ALT)*

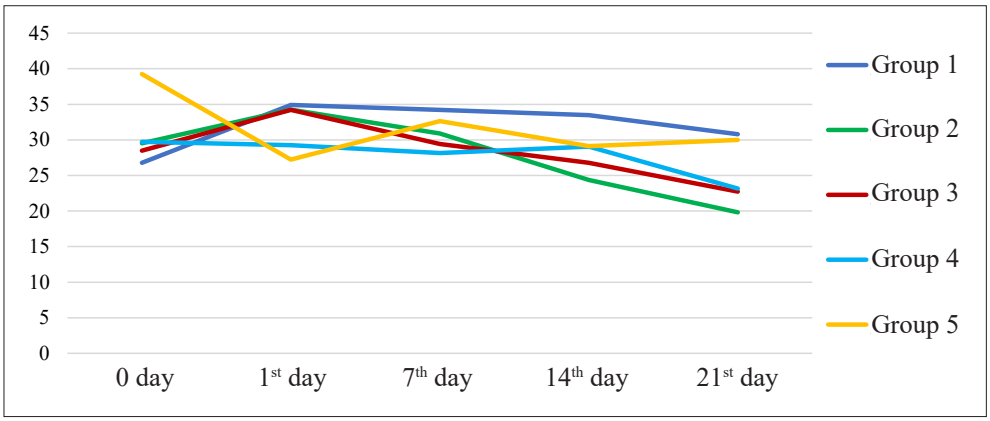


***Figure 10**. The graphic evaluation of the albumin*

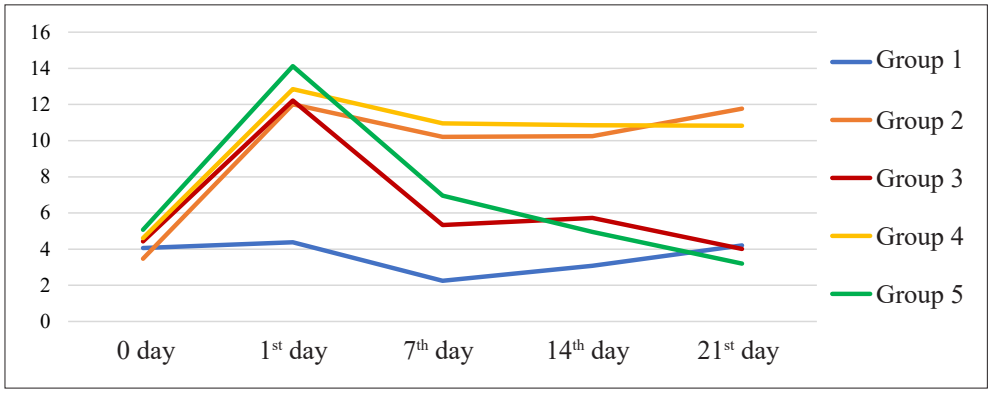


Figure 11. *The graphic evaluation of bilirubin*

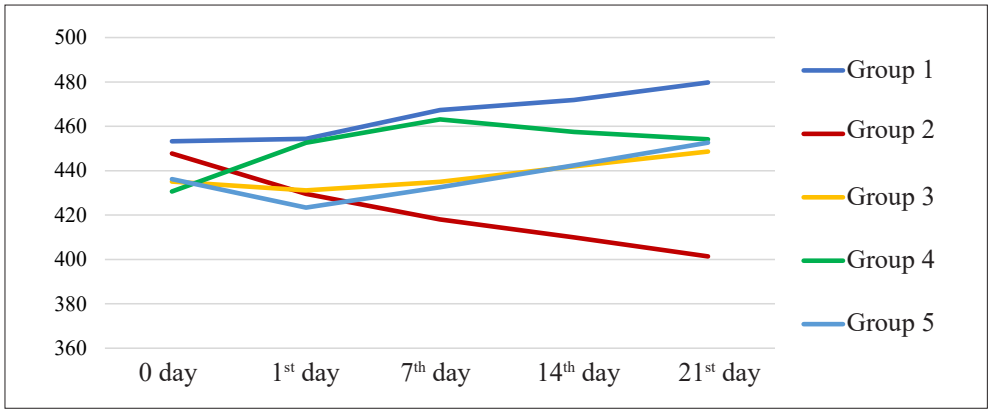


Figure 12. *The graphic evaluation of weight*

Evaluation of histological preparations. At the preparation staining with Hematoxylin and Eosin the second group preparations were compared with those in the group 3 (Fig. 13, 14), and the group 4 – after hepatic damage with TAA with the group 5 (Fig. 15, 16). The liver tissue after resection in the group 2 is incompact and congested at several sites compared to the group 3.

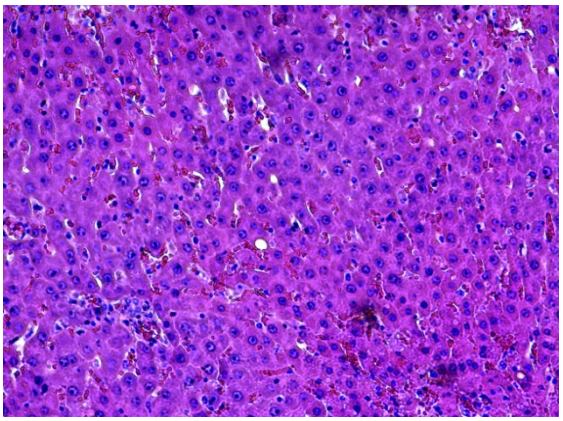


Figure 13. *Liver tissue (group 2) magnification 100x*

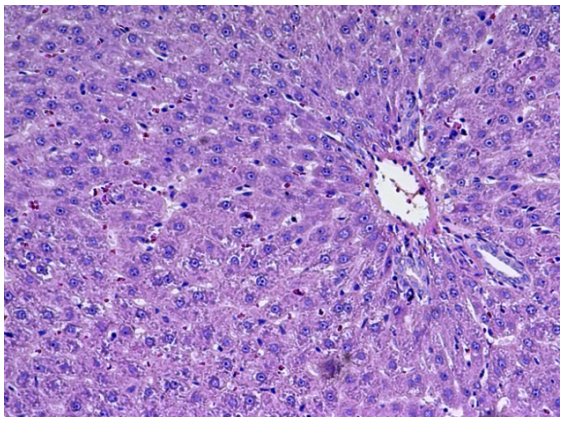


Figure 14. *Liver tissue (group 3) 100x*

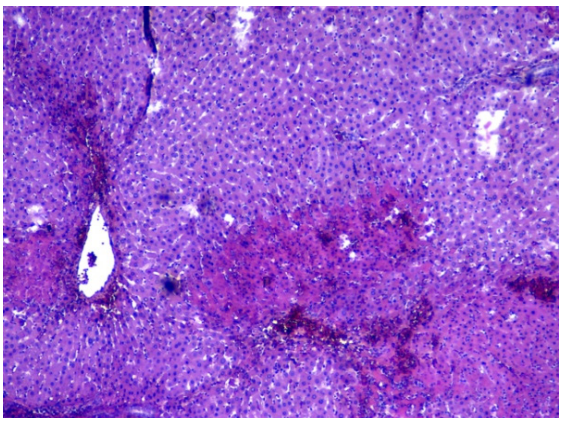


***Figure 15**. Liver tissue (group 4) magnification 40x*

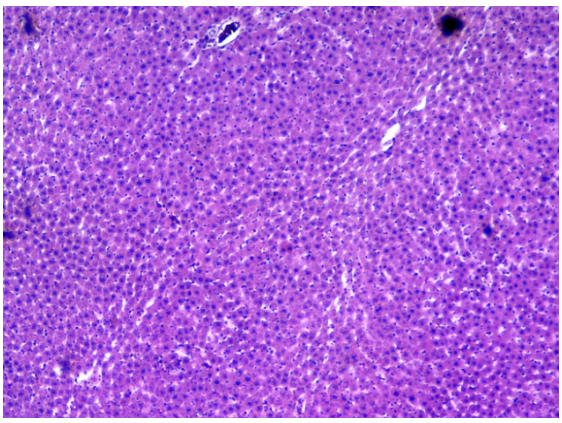


***Figure 16**. Liver tissue (group 5) 40x*

The evidence of the presence and transfer of stem cells using the electron microscope. After administration of mesenchymal stem cells to experimental animals, we observed the penetration of these cells and their subsequent growth. The mesenchymal stem cells (MSC) inoculated into the damaged liver transferred from the site of administration through the vena portae to all areas of the liver. The nuclei of all cells present in the liver are blue-shining, and the MSCs nuclei are red shining (Fig. 17, 18).

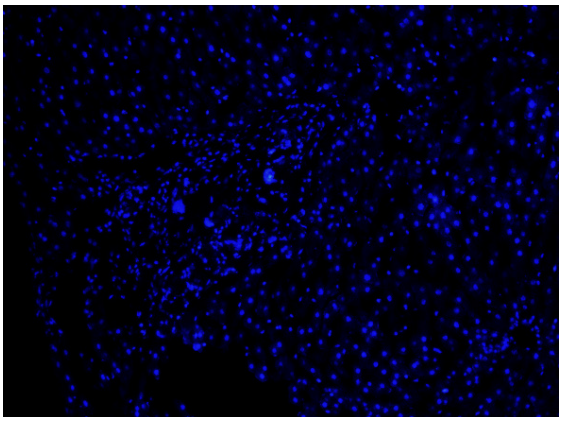


***Figure 17**. Displayed nuclei of all cells of the liver*

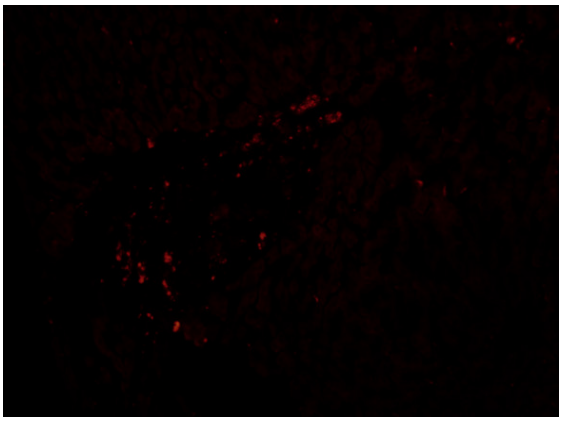


***Figure 18**. Nuclei of MSCs in the liver*

The liver has permanently attracted people's attention, the Greeks have already recognized the inexhaustible feature of the liver-regeneration, whose principles have still being studied [14]. The liver has an irreplaceable role in the living organism. Like other organs, it is subject to various diseases: genetic, traumatic, inflammatory, and cancerous diseases. There are several types of tumours in the liver, both benign and malignant. The most appropriate healing method for liver tumours, including carcinomas, is radical resection of the liver. Hepatic carcinoma often occurs in the cirrhotic liver, thus the functional capacity of the left cirrhotic remains of the liver is significantly reduced that increases the mortality and shorten the patient survival time [15]. A serious problem with liver cancer is therefore the possibility of performing radical surgical resection and leaving sufficient functional parenchyma of the liver. To support the liver regeneration after extensive resections, the possibility of treatment with the application of mesenchymal stem cells is being investigated [9; 16].

Stem cells are primarily undifferentiated cells capable of self-regeneration and differentiation to other, more specialized cell types. In the experiment, mesenchymal stem cells (MSCs) were applied to experimental animals. The bone marrow mesenchymal stem cells with differentiation potential to four lineages (mesenchymal and vascular smooth muscle lineages), and stromal and immunomodulatory capacities, the stem cell attributes are multipotentiality, self-renewal, tissue regeneration, population heterogeneity, plasticity. These stem cells are relatively easy to isolate, the source for their isolation is well available, the cells proliferate well in the cultures, but the resulting population is considerably heterogeneous [17; 18]. The experimental studies on mice by M. Kaibori et al. [10] attempted to stimulate the liver regeneration by transplanting MSCs taken from the bone marrow of mice. Autologous transplantation of MSCs has shown a great promise to improve the tissue regeneration in various acute and chronic diseases. The treatment has shown improvement in the liver regeneration in acute

and chronic forms of the disease in both preclinical studies and pilot clinical trials. The stem cell therapy can provide effective treatment and facilitate regeneration after resection of the liver, or before resection. The study by M. Kaibori et al. 2012 [10] explained the important role of stem cells in the liver regeneration after extensive resection of the liver.

CONCLUSIONS

Based on the available literature and various clinical and preclinical studies, we have also progressed in our experiment. The stimulation effect of MSCs on the liver regeneration was observed based on the laboratory values of alanine aminotransferase (ALT), bilirubin, and albumin, control of the experimental animal weight, and histology. The results obtained were identical to those used in the literature. A statistically significant decrease in the ALT ($p<0.001$) and bilirubin ($p<0.001$) values in the groups of the rats treated with stem cells was observed and compared to the groups without stem cell transplantation. As well, a statistically significant increase in albumin in the groups after application of mesenchymal stem cells was found, $p<0.001$. The results of the experiment lead to the conclusion that mesenchymal stem cells have a significant effect on the liver regeneration after its damage. The electron microscopy revealed the transfer of mesenchymal stem cells from the application site to the site of their action and differentiation. In the future, it is worthwhile to examine the other properties of stem cells, to continue experimental studies and then to apply this knowledge to the medical practice. Stem cells represent a major advance in the treatment of liver tumours and stimulation of the liver regeneration.

REFERENCES

[1] Hrubiško, M. (2004). Therapeutic application of (blood) stem cells 1. Part – History and division. *Internal Medicine*, 4(9), 466-470.

[2] Levendecker, A., Pinheiro, C.C.G., Amano, M.T., & Bueno, D.F. (2018). The use of human mesenchymal stem cells as therapeutic agents for the in vivo treatment of immune-related diseases: A systematic review. *Frontiers in Immunology*, 9, article number 2056.

[3] Wobus, A.M., & Boheler, K.R. (2005). Embryonic stem cells: Prospects for developmental biology and cell therapy. *Physiological Reviews,* 82, 635-678.

[4] Fialova, M., Brychta, I. (2012). Stem cells in surgery. *Slovak Surgery,* 9, 48-51.

[5] Fausto, N. (2004). Liver regeneration and repair: Hepatocytes, progenitor cells and stem cells. *Hepatology,* 39, 1477-1487.

[6] Itaba, N., Noda, I., Oka, H., Kono, Y., Okinaka, H., Yokobata, T., Okazaki, S., Morimoto, M., & Shiota, G. (2018). Hepatic cell sheets engineered from human mesenchymal stem cells with a single small molecule compound IC-2 ameliorate acute liver injury in mice. *Generative Therapy,* 9, 45-57.

[7] Sia, D., Villanueva, A., Friedman, S.L., & Llovet, J.M. (2017). Liver cancer cell of origin, molecular class, and effects on patient prognosis. *Gastroenterology*, 152, 745-761.

[8] Lee, M.W., & Lim, H.K. (2018). Management of sub-centimeter recurrent hepatocellular carcinoma after curative treatment: Current status and future. *World Journal of Gastroenterology,* 24, 5215-5222.

[9] Hackl, Ch., Schlitt, H.J., Renner, P., & Lang, S.A. (2016). Liver surgery in cirrhosis and portal hypertension. *World Journal of Gastroenterology*, 22, 2725-2735.

[10] Kaibori, M., Adachi, Y., Shimo, T., Ishizaki, M., Matusi, K., Tanaka, Y., Ohisi, M., Araki, Y., Okumura, T., Nishizawa, M., & Kwond, A.H. (2012). Stimulation of liver regeneration after hepatectomy in mice by injection of bone marrow mesenchymal stem cells via the portal vein. *Transplantation Proceedings,* 44, 1107-1109.

[11] Higgins, G.M., & Anderson, R.M. (1931). Experimental pathology of the liver-restoration of the liver of the white rat following partial surgical removal. *Archive of Pathology*, 12, 186-202.

[12] Tse-Min, C.H., Yi-Maun, S., Ru-Ping, L., Tzyy-Wen, Ch., & Bang-Gee, H. (2008). Single dose intravenous thioacetamide administration as a model of acute liver damage in rats. *International Journal of Experimental Pathology*, 89, 223-231.

[13] Koblihová, E., Mrázová, I., Vernerová, Z., & Ryska, M. (2014). Acute liver failure induced by thioacetamide: Selection of optimal dosage in wistar and lewis rats. *Physiology*, 63, 491-503.

[14] Tsoucalas, G., Kapsoritakis, A., Potamianos, S., & Sgantzos, M. (2017). Promethean and Tityean hepatic regeneration, unveiling hepar's myth. Post mortem observation, surgical ascertainment, or just fiction? *Journal of Universal Surgery,* 5, article number 2.

[15] Klener, P., & Brodanová, M. (2006). Diseases of Liver. In *Internal Medicine* (pp. 629-698). Praha: Galén.

[16] Michalopoulos, G.K. (2017). Hepatostat: Liver regeneration and normal liver tissue maintenance. *Hepatology,* 65, 1384-1392.

[17] Muraglia, A., Cancedda, R., & Quarto, R. (2000). Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. *Journal of Cell Science,* 113, 1161-1166.

[18] Charbord, P. (2010). Bone marrow mesenchymal stem cells: Historical overview and concepts. *Human Gene Therapy*, 21, 1045-1056.

Стимуляція регенерації печінки за допомогою мезенхімальних стовбурових клітин кісткового мозку

Мартіна Відова Угурбаш[1,2], Яна Катюхова[1,2], Дарина Петрашова[1], Тімея Спакова[1], Йозеф Радонак[1,2]

[1]Університет Павла Йозефа Шафарика в Кошице
040 11, Trieda SNP 1, м. Кошице, Словацька Республіка

[2]Університетська лікарня імені Л. Пастера в Кошице
040 11, Trieda SNP 1, м. Кошице, Словацька Республіка

Анотація. Основною метою даного експерименту було довести вплив мезенхімальних стовбурових клітин кісткового мозку (МСК) на стимуляцію регенерації печінки. Приділено увагу адаптації стовбурових клітин до нового середовища та їх перенесення в анатомічні структури. Експеримент включав 40 самців щурів Спрег-Доулі (СД) віком від 10 до 12 тижнів. Біомоделі були розділені на п'ять груп у однаковій кількості (n=8). Група 1 складалася з контрольної проби з восьми здорових щурів. 2 група складалася з восьми щурів після резекції печінки без застосування МСК. 3 група була після резекції печінки та застосування МСК. Група 4 – після ураження печінки індукують тіоацетамідом (ТАА), без трансплантації МСК. 5 група була після хімічного ураження печінки введенням ТАА та трансплантацією МСК. Процес стимуляції печінки спостерігали на підставі лабораторних показників аланінамінотрансферази (АЛТ), альбуміну та білірубіну. Також порівнювали вагу щурів у кожній групі. Тварин забивали через 1 день, 7 днів, 14 днів і 21 день. У нашому експерименті було виявлено статистично значуще зниження рівня АЛТ (P≤0,001) і білірубіну (P≤0,001) у групах 3 і 5 (лікування МСК) порівняно з групами без МСК (групи 2 і 4). Підвищення рівня альбуміну в групах 3 і 5 було статистично значущим. Результати цього експерименту привели до висновку, що трансплантація МСК має важливий ефект для лікування та стимуляції регенерації печінки після травми. Введення МСК може бути надзвичайно корисним у ряді клінічних застосувань у лікуванні пухлин печінки. Це дозволить виконати обширну резекцію печінки без ризику печінкової недостатності

Ключові слова: стовбурові клітини, тіоацетамід, резекція печінки, регенерація печінки