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A CASE REPORT ON INTRAPORTAL INJECTION OF AUTOLOGOUS BONE MARROW-DERIVED MONONUCLEAR CELLS AND LIVER TRANSPLANTATION IN A PATIENT WITH CIRRHOSIS

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To date, liver transplantation remains the only effective treatment for patients with cirrhosis. Due to lack of other effective, alternative therapeutic methods, the search and development of new treatment technologies is problem number one. The development of cellular technologies is promising for use in clinical practice. Using this observation as an example, the safety and efficacy of cell therapy technology for prolonged stay on the liver transplant waiting list by a patient with cirrhosis is shown. After intraportal injection of autologous bone marrow-derived mononuclear cells, liver cirrhosis stabilized on the CTP and MELD-Na scales for 22 months of observation, which allowed the patient to wait for an organ and successfully undergo liver transplantation.

Keywords: autologous bone marrow-derived mononuclear cells, cell therapy, stem cells, intraportal injection, bone marrow, portal flowmetry, liver cirrhosis, liver transplantation.

INTRODUCTION

Liver cirrhosis (cirrhosis) is a terminal state of a chronic disease caused by various etiological factors, accompanied by severe inflammation, hepatocyte necrosis and liver fibrosis. This condition progresses irreversibly, leading to decompensation and death [1–3]. Currently, orthotopic liver transplantation (OLT) is the only radical treatment for cirrhosis [4–6].

However, in the Russian Federation, as in other countries, there is significant organ shortage and, as a result, higher number of waitlisted candidates [7, 8].

Thus, under limited transplant care and lack of effective drugs that increase the synthetic liver function, the search for and development of new technologies for treatment and support of patients with end-stage liver disease is an urgent task. This problem is relevant, including for patients who have been waitlisted for OLT. According to clninicaltrials.gov, 104 (26 completed) clinical trials using stem cells (SCs) in the treatment of liver diseases were conducted in 2020. However, reports on the method of SCs administration in cirrhosis are contradictory [9–12]. The authors describe both a simplified version of administration – into the peripheral vein, and an intra-arterial, intra-portal route of administration. In our observation, in a patient with end-stage cirrhosis, intraportal administration of SCs to achieve increased concentration in the liver was tested.

OBSERVATION

***Female patient**, 54 years old, diagnosed with cryptogenic hepatitis, cirrhosis, was included in the waiting list for liver transplantation in July 2015 at the Granov Russian Scientific Center of Radiology and Surgical Technology, St. Petersburg, Russia. While on the waitlist, she received standard drug therapy (hepatoprotectors, diuretics), while her liver function progressively deteriorated.*

In 2017, intraportal infusion of autologous bone marrow-derived mononuclear cells was proposed during a planned visit. Prior permission from the local ethics committee of the clinic was obtained. After signing the informed consent, the patient was examined (Table 1). The patient's initial status was assessed: Child-Turcotte-Pugh class B, MELD-Na 15. Contrast-enhanced spiral CT imaging of the chest, abdomen, and pelvis was performed, the liver vessels were assessed. No ascites and portal vein thrombosis were observed on tomograms. The recipient's baseline quality of life was assessed using the SF-36 questionnaire. The physical and psychological health scores were 45.14 and 41.55, respectively. Based on the data obtained, it was established that the patient had no contraindications according to the inclusion criteria (liver cirrhosis) and exclusion (no malignant neoplasm, portal vein thrombosis, active bacterial, viral infection) to the cell therapy procedure (Russian Federation patent RU2671560 C1 of November 2, 2018).

Subsequently, under general anesthesia in the operating room, 256 ml autologous bone marrow was aspirated from the posterior iliac crest using a 4G × 70 mm needle (TSUNAMI MEDICALL Italy).

After the bone marrow extraction procedure, the aspirate was sent in a transfusion bag in a thermocontainer to the laboratory, where mononuclear cell (MNC)

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isolation was carried out using an automated Maco Press Smart separator (Maco Pharma, France). We obtained 51.8 mL of MNC suspension. Then, we performed a qualitative analysis of MNCs and hematopoietic cell population with assessment of their viability using flow cytometry (Beckman Coulter, USA). The result is shown in Fig. 1. As an MNC medium, we used a 0.9% NaCL solution, a 20% human albumin solution, and 2500 IU of heparin solution. The cells were resuspended to a final volume of 93.8 mL.

About 2.5 hours after MNCs were obtained in the X-ray operating room under local anesthesia using ultrasound and X-ray navigation, the portal vein (PV) was punctured with a 22G needle (Cook Medical, USA). After removing the mandrel and obtaining blood from the lumen of the needle on a 0.35G-shaped guidewire

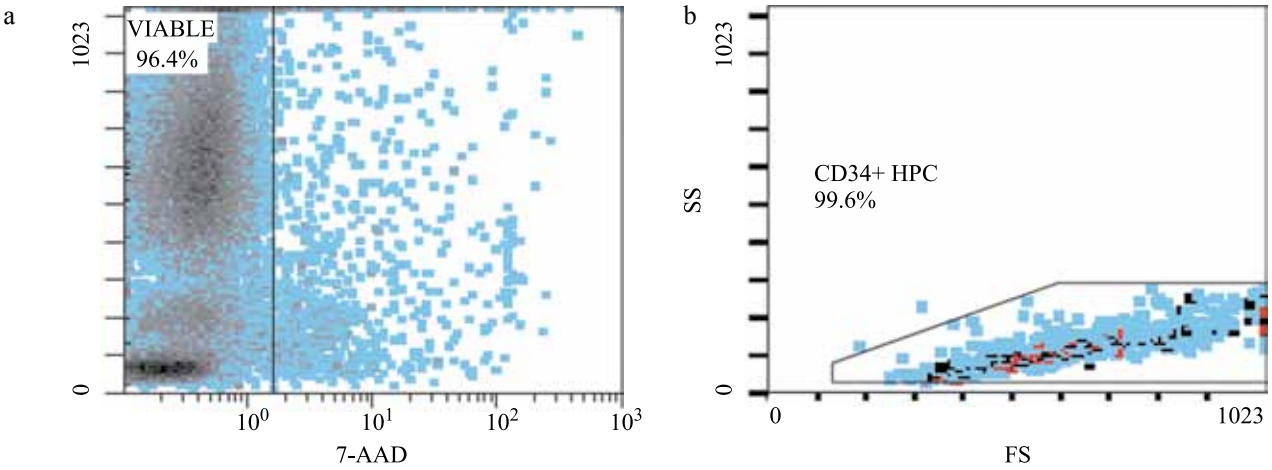

Fig. 1. Result of MNC suspension viability assessment by flow cytometry; a) viability of all MNC cells is 96.4%; b) viability of hematopoietic stem cells (CD34+) in MNC suspension is 99.6%

Table 1

Assessment of complete blood count and biochemistry panel results during the first 12 month of follow-up

	Before treatment	2 mths	4 mths	6 mths	8 mths	10 mths	12 mth	Units
White blood cells (WBC)	4.29	4.08	4.59	4.29	4.64	4.67	4.41	10^9/L
Red blood cells (RBC)	3.9	4.14	4.49	4.6	4.78	4.63	4.32	10^{12}/L
Hemoglobin (HGB)	109	113	127	127	131	128	135	g/L
Hematocrit (HCT)	33.1	34.4	38.8	38.9	38.3	39.4	37.1	%
Platelets (PLT)	88	77	83	101	104	95	105	10^9/L
Absolute neutrophil count (ANC)	2.27	2.14	2.65	2.52	2.75	2.62	2.51	10^9/L
Absolute lymphocyte count (LYMPH)	1.29	1.15	1.15	0.98	1.15	1.22	1.04	10^9/L
Absolute monocyte count (MONO)	0.46	0.57	0.6	0.57	0.5	0.61	0.51	10^9/L
Absolute eosinophil count (EO)	0.21	0.18	0.15	0.18	0.2	0.17	0.29	10^9/L
Absolute basophil count (BASO)	0.06	0.04	0.04	0.04	0.04	0.05	0.06	10^9/L
Glucose	5.55	5.42	5.43	5.95	5.57	6.97	5.26	mmol/L
Urea	2.7	3.3	3.9	3.6	3.8	4.3	4.5	mmol/L
Creatinine (Crea)	54	49.2	53.1	50.6	53.8	57.8	66.1	μmol/L
Total bilirubin	50	38.6	40.8	37.9	34.3	40.2	37.7	μmol/L
Direct bilirubin	28.4	20.2	20	17.9	16.7	16.6	15.5	μmol/L
AST	41	46	49	38	35	37	37	U/L
ALT	17	23	24	19	14	16	19	U/L
ALP	223	196	232	237	228	191	213	U/L
Potassium (K)	3.7	4.3	4.2	4.2	4.3	4.2	4.6	mmol/L
Sodium (Na)	141	139	142	140	137	139	142	mmol/L
Total protein	74	71	75	72	70	72	78	g/L
Albumin (ALB)	31	31	33	33	32	33	34	g/L
Quick prothrombin (Factor II)	53	56	57	53	58	56	55	%
Prothrombin time (PT)	18.4	17.8	17.7	18.2	17.2	17.5	18.8	sec.
INR	1.55	1.49	1.48	1.55	1.44	1.48	1.53	

(Starter, Boston, USA), a 4 F dilatation catheter (1 F = 0.33 mm, Cook Medical, USA) was inserted and angiography was performed from the v. portae with 15–20 mL of a water-soluble contrast agent (Omnipaque 350, Nycomed, USA). Next, a straight aortic catheter with 5 F lateral orifices (Cook Medical, USA) was placed on a metal guide (Storq, Cordis, USA). Taking into account the presence of collaterals caused by portal hypertension, before injecting the cell suspension, portal doppler flowmetry was performed using an automatic injector with 15–20 mL of a water-soluble contrast agent. The injection was performed at 0.5 mL/sec, 0.8 mL/sec, 1.0 mL/sec and 1.5 mL/sec to determine the optimal rate of selective perfusion of PV segmental branches. At 0.5 mL/sec injection rate, the PV segmental branches were not visualized, the contrast agent was discharged through the extrahepatic PV collaterals. At 0.8 mL/sec injection rate, uniform contrasting of all the PV segmental branches was achieved and the absence of blood flow along the collaterals was noted (Fig. 2). A 93.8 mL MNC suspension was injected from the portal vein bifurcation level at 0.8 mL/sec without loss of cells along the collaterals. The puncture canal was sealed with a hemostatic sponge, followed by removal of the catheter and fixation of aseptic dressing.

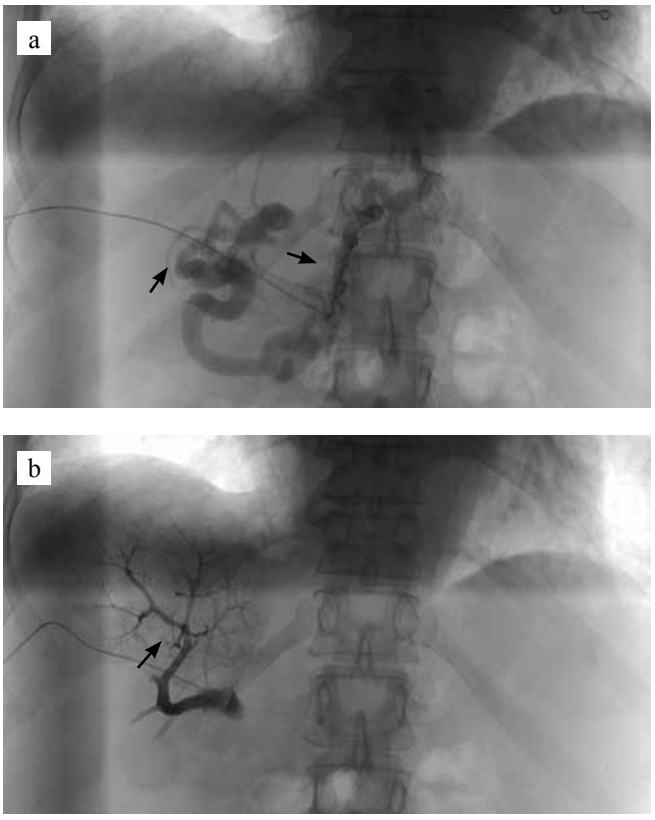

Fig. 2. Angiograms of the patient. a) portogram with contrast agent injection at the rate of 1 mL/sec and 1.5 mL/sec: the portal vein trunk and collaterals were visualized (black arrows); b) portogram with contrast agent injection at a rate of 0.8 mL/sec: only the portal vein segmental branches were perfused (optimal speed)

Ultrasound examination was performed after the operation. It found no bleeding at the manipulation site. There were no complications and clinically significant adverse events in the early postoperative period during the follow-up of the patient. The patient was discharged after 48 hours of hospital observation and control blood tests.

Then, every 2 months, the liver function indices were assessed according to the MELD-Na and CTP scales (Table 2), quality of life (Table 3).

During 22 months of follow-up, the patient's liver functional parameters improved: total bilirubin and hepatic transaminases levels decreased, albumin serum levels increased. The degree of liver failure according to CTP scale (7 points) and MELD-Na (14 points) was stabilized. The patient's quality of life indicators improved according to the SF-36 questionnaire (physical health – 56.44 points, mental health – 53.25 points).

Morphological assessment before the introduction of cells showed pronounced leukocytic infiltration, inflammation; the number of binuclear cells was 12 cells/mm^2, no mitoses were detected (Fig. 3). According to According to immunohistochemistry (IHC), the Ki67 marker expression was 2%, alpha-fetoprotein was not expressed.

Two months after the use of cell therapy, we performed repeated trepan biopsy of the liver from the same segment with subsequent morphological and IHC assessment (Fig. 5). There was decreased inflammatory infiltration, a significant increase in the number of binuclear

Table 2

Assessment of liver failure based on MELD-Na and CTP scales

Observation period	MELD-Na, points	Child-Turcotte-Pugh, points
Before treatment	15	Class B – 8
2 months	14	Class B – 8
4 months	15	Class B – 8
6 months	14	Class B – 7
8 months	14	Class B – 7
10 months	14	Class B – 7
12 months	15	Class B – 8
18 months	14	Class B – 7
22 months	14	Class B – 7

Table 3

Quality of life assessment (SF-36 questionnaire)

Observation period	Physical health	Mental health
Before treatment	45.14	41.55
1 month	47.00	45.00
4 months	50.53	48.48
8 months	54.43	52.09
12 months	54.77	39.78
18 months	51.10	46.11
22 months	56.44	53.25

cells from 12 to 19 cells/mm^2. No mitoses were detected. Ki67 expression was 3% (no significant dynamics), alpha-fetoprotein was not expressed.

After 22 months of follow-up after MNC was injected, the patient underwent OLT from a postmortem donor using the piggyback implantation technique with preservation of the retrohepatic section of the recipient's inferior vena cava.

There were no post-transplant complications. Graft function was satisfactory. The patient is alive and regularly monitored at the outpatient transplant center.

DISCUSSION

OLT remains the main radical treatment for cirrhosis patients. A longer wait for donor organ entails a higher waitlist mortality. Prior to OLT, patients receive only drug therapy in order to correct blood liver function. The lack of competitive effective therapy prompts the search for other ways to solve this problem. Regenerative medicine and cell therapy may serve as the most likely method of reducing liver failure mortality.

The safety and efficacy of stem cells in cirrhosis has been proven in clinical trials in a limited sample of patients [11–14]. However, in the process of human stem cell therapy, researchers face three main questions: how to obtain SCs, the administration-delivery route, and the choice of a cell population to achieve the best therapeutic effect.

In our case, the source of SCs was autologous bone marrow-derived mononuclear cells obtained from a patient with cirrhosis. Apparatus separation of the MNC made it possible to obtain a cell suspension with high viability.

According to a literature review, the authors have not previously detailed and substantiated the rate of cell administration and the advantages of the portal route of administration in the presence of venous collaterals [15–18].

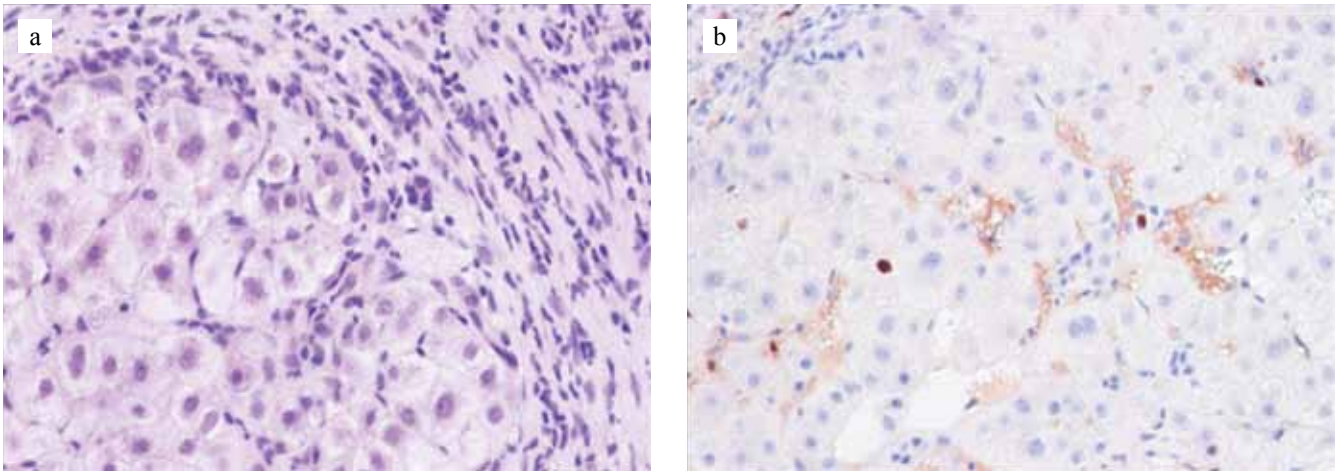

Fig. 3. Histological examination of liver biopsies before MNC infusion: a) infiltration by WBC, inflammation; b) IHC, expression of the Ki67 marker – 2%, alpha-fetoprotein was not expressed

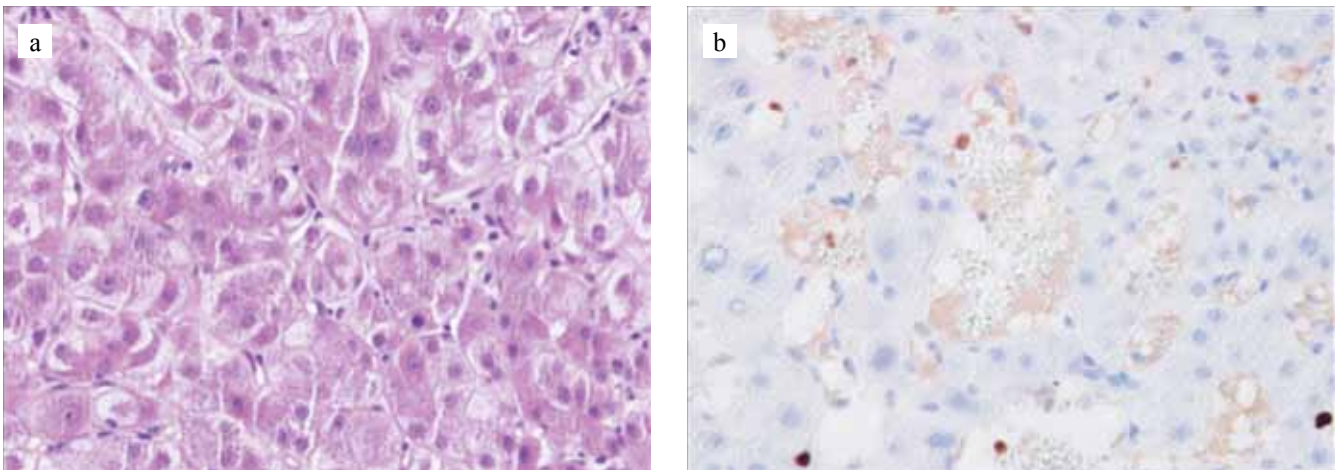

Fig. 4. Histological examination of liver biopsies after MNC infusion (2 months): a) infiltration by WBC and inflammation decreased, the number of binucleated cells increased (19 cells/mm^2). b) IHC, expression of the Ki67 marker – 3%, alpha-fetoprotein was not expressed

In the presented clinical case, we have demonstrated the portal doppler flowmetry technique, which allows to prevent the loss of cell suspension through the portosystemic venous collaterals when administered via the portal vein.

CONCLUSION

This proven cell therapy technology is safe and allows to improve liver function and stabilize the course of cirrhosis according to prognostic scales CTP (from 8 to 7), MELD-Na (from 15 to 14) while on the OLT waiting list.

The study on targeted delivery of a cell suspension to the liver through the portal vein suggests the need for a personalized approach in the use of cell therapy. A further randomized study on an enlarged sample of patients will be of great scientific interest.

The authors declare no conflict of interest.

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