80887_Auto_Edited-Check.docx

WORD COUNT	3271	TIME SUBMITTED	01-MAR-2023 04:23PM
		PAPER ID	97139934

Endoscopic ultrasound-guided intraportal injection of autologous bone marrow in patients with decompensated liver cirrhosis: A pilot study

Shao-Peng Zheng, Ao-Jian Deng, Jing-Jing Zhou, Ling-Zhi Yuan, Xiao Shi, Fen Wang

Abstract

BACKGROUND

Recently, stem cell therapy has been extensively studied as a promising treatment for decompensated liver cirrhosis (DLC). Technological advances in endoscopic ultrasonography (EUS) have facilitated EUS-guided portal vein (PV) access, through which stem cells can be precisely infused.

AIM

To investigate the feasibility and safety of fresh autologous bone marrow injection into the PV under EUS guidance in patients with DLC.

METHODS

Five patients with DLC were enrolled in this study after obtaining their written informed consent. EUS-guided intraportal bone marrow injection with a 22G FNA-needle was performed using a transgastric, transhepatic approach. Several parameters were assessed before and after the procedure for a follow-up period of 12 mo.

RESULTS

Four males and one female with mean age of 51 years-old participated in this study. All patients had hepatitis B virus-related DLC. EUS-guided intraportal bone marrow injection was performed in all patients successfully without any complications such as hemorrhage. The clinical outcomes of the patients revealed improvements in clinical

symptoms, serum albumin, ascites, and Child-Pugh scores throughout the 12-mo follow-up.

CONCLUSION

The use of EUS-guided fine needle injection for intraportal delivery of bone marrow was feasible, safe and appeared effective in patients with DLC. This treatment may thus be a safe, effective, non-radioactive, and minimally invasive treatment for DLC.

Key Words: Endoscopic ultrasonography; Fine needle injection; Portal vein; Decompensated liver cirrhosis; Bone marrow

Zheng SP, Deng AJ, Zhou JJ, Yuan LZ, Shi X, Wang F. Endoscopic ultrasound-guided intraportal injection of autologous bone marrow in patients with decompensated liver cirrhosis: A pilot study. *World J Gastrointest Surg* 2023; In press

Core Tip: In this manuscript, we show that the use of endoscopic ultrasonography (EUS)-guided fine needle injection for intraportal delivery of stem cells was feasible, safe and appeared effective in patients with decompensated liver cirrhosis (DLC). And it is the first attempt to investigate the feasibility and safety of fresh autologous bone marrow injection into the portal vein under EUS guidance in patients with DLC.

INTRODUCTION

Cirrhosis refers to a late stage of liver fibrosis caused by chronic liver damage due to various etiologies such as alcohol and viral hepatitis. Liver cirrhosis (LC) is the 11th most common cause of death world-wide[1]. The most frequent clinical manifestations of hepatic decompensation include ascites, variceal bleeding, hepatic encephalopathy, and jaundice. The prognosis of these patients is worse and significantly shorter than that of patients with compensated cirrhosis[2]. Despite various medical therapies, the morbidities and mortality associated with decompensated LC (DLC) is high. To date,

liver transplantation is the only curative treatment for DLC. However, the shortage of donor livers, immunological rejection, surgical complications and high cost greatly limit the clinical application of liver transplantation.

In recent years, stem cell therapy has been extensively studied as a promising treatment for DLC. Stem cells can not only differentiate into hepatocytes, but also play an important role in anti-fibrosis, anti-inflammatory and the immune regulation of liver diseases[3-7]. Thus, stem cell therapy has the potential to restore normal liver function by increasing the number of normal hepatocytes and improving the pathological structure of liver tissue. However, the application of this therapy has been hindered due to the genomic instability and the tumorigenicity of stem cells[8,9].

Strategies such as liver-targeted stem cell therapy can not only increase the number of therapeutic stem cells in the liver but also reduce the distribution of stem cells in non-targeted organs and the total amount of infused stem cells, and therefore reduce the risk of malignant transformation of stem cells. With the recent technological advancements in endoscopic ultrasonography (EUS) and its instrumentation, EUS offers a potential access to the portal vein[10-12], through which the stem cells can be precisely delivered to the liver. Due to the proximity of the portal vein to the gastrointestinal tract, EUS-guided access to the portal venous system has been studied as an alternate approach to standard routes for portal vein angiography and portal pressure gradient measurement. To date, data on EUS-guided intraportal fine needle injection (FNI) of stem cells or bone marrow in patients with DLC remain scarce. In this prospective study, we performed fresh autologous bone marrow injection into the portal venous system of patients with DLC under the guidance of EUS and evaluated its safety and efficacy at 12 mo of follow-up.

MATERIALS AND METHODS

In this study, patients with DLC were enrolled for EUS-guided intraportal autologous bone marrow infusion between January 2020 and February 2020. This study was reviewed and approved by the institutional ethics committee of the local hospital (No:

2018-S403; date: December 26, 2018). Registration number of this study was ChiCTR2000035269 in Chinese Clinical Trial Registry. Written informed consent was obtained from all enrolled patients. All methods related to this study were carried out in accordance with the ethical standards of the declaration of Helsinki concerning research involving human subjects. Patients satisfying the following criteria were enrolled in this study.

Inclusion criteria

Inclusion criteria: (1) Age: > 18 years; (2) decompensated liver cirrhosis with ascites; (3) etiology: alcoholic or posthepatitic cirrhosis; and (4) endoscopy was tolerable after anesthesia evaluation.

Exclusion criteria

Exclusion criteria: (1) Pregnant or lactating women; (2) patients with severe anemia and coagulation dysfunction (international normalized ratio > 1.5); (3) failure of hemostasis treatment for gastrointestinal bleeding in the past month; (4) presence of spontaneous peritonitis, hepatic encephalopathy, hepatorenal syndrome and acute infection; (5) presence of coexisting severe heart, lung, kidney, blood system or other diseases, history of mental illness; and (6) malignant tumor of the liver or other organs.

Therapeutic methods

Bone marrow sampling: All patients were given the standard medical treatment for LC and under antiviral therapy against hepatitis B after hospitalization. After preoperative evaluation and enrollment in this study, the autologous bone marrow sampling was performed. The aspiration of bone marrow were performed in the sterile operating room. The right posterior superior iliac spine was selected as the puncture site, with skin was cleaned with 70% alcohol. The skin, subcutaneous tissues, and periosteum overlying the selected site for puncture were infiltrated with local anesthesia, and serial punctures from multiple sites were performed. With needles passed perpendicularly

into the cavity of the ileum at a point just right posterior superior iliac spine , about 30 mL of the patients' bone marrow was collected in a syringe containing 10 mL of heparin saline (62.5 U/mL). And the bone marrow samples were subjected to transplantation immediately after aspiration in the same sterile operating room. In addition, 1 mL of bone marrow was collected for flow cytometry to count the total number of nucleated cells and the proportion of $CD34^+$ cells, which have the character of plasticity and can change into hepatocytes.

Endoscopic procedure: The EUS-guided portal vein puncture was performed by experienced endosonographers using an endoscopic ultrasound system (EU-ME2, Olympus, Tokyo, Japan), a linear echoendoscope (UCT-260, Olympus, Tokyo, Japan), and a 22G FNA-needle (Cook Medical, Winston-Salem, NC, United States). Firstly, under intravenous anesthesia with propofol, the echoendoscope was introduced into the stomach transorally. Then, after identification of the portal vein (PV), the endoscopic FNA needle was advanced through the liver parenchyma into the lumen of the PV under Doppler imaging. After puncturing the intrahepatic PV, the stylet was withdrawn from the needle and blood was aspirated before injection to confirm the position of the needle. Then, through the FNA needle, a total of 40 mL of fresh autologous bone marrow fluid (30 mL of fresh autologous bone marrow and 10 mL of heparin saline) was injected into the PV at an approximate rate of 1 mL/min under continuous ultrasonic monitoring. Needle placement was meticulously monitored during injection to ensure consistency. It usually takes about 30-40 min for the whole injection, and the injection is administered under the guidance of endoscopic ultrasound, which helps us to maintain the infusion rate and needle stability. Following completion of the infusion, the FNA needle was gradually removed. Upon withdrawal of the needle, just prior to leaving the liver capsule, Color Doppler imaging was used to ensure that there was no flow in the needle track. Finally, the needle was removed, followed by compression at the puncture site for about 5 min using the ultrasonic stylet. Before withdrawing the echoendoscope, intrahepatic or perihepatic hemorrhage or

hematoma was ruled out by Color Doppler. Subsequently, the patient was placed under close observation and the ongoing medical treatment was continued.

Follow-up

The endpoint of the follow-up period was 12 mo after the procedure. During the follow up visits, clinical history was collected and a physical examination, laboratory tests and abdominal ultrasonography were performed. The main clinical symptoms noted were the presence/absence of ascites, variceal bleeding, hepatic encephalopathy and jaundice. Laboratory tests included a complete hemogram, liver function tests, coagulation profile, serum hyaluronic acid, serum laminin, serum collagen IV and serum procollagen III. Abdominal ultrasound emphasized the grade of ascites, the PV diameter, portal vein thrombosis, and neoplastic lesions in the liver. At the same time, elastography was performed by ultrasound to estimate the liver stiffness (LS) and the fat attenuation parameters.

Statistical analysis

The quantitative variables are described as mean ± SD. The changes in the parameters relative to baseline at 1 mo, 3 mo, 6 mo, and 12 mo after treatment were determined using an analysis of variance (ANOVA) with Fisher's protected least-significant difference test. The IBM® SPSS® for Windows version 25.0 software was used for statistical data analysis.

RESULTS

Baseline characteristics

In this study, 5 patients (4 males and 1 female) were included. The etiology of cirrhosis in all patients was hepatitis B virus infection. The mean age of the patients was 51 (range 30-71) years. The main clinical symptoms were abdominal distention (3/5), gastrointestinal bleeding (1/5), edema (1/5), and abdominal pain (1/5). The total number of nucleated cells in 30 mL bone marrow was 300-500 × 10^6 and the percentage

of $CD34^+$ cells was 0.52%-1.73%. Detailed characteristics are shown in Table 1. The follow-up period was 12 mo.

Feasibility

The intrahepatic part of the PV could be clearly demonstrated by EUS in all patients. Access to the targeted vessel was accomplished without any failures. All patients successfully underwent EUS-guided FNI into the PV with a 22G FNA-needle. The precise delivery of a total of 40 mL of bone marrow fluid to the liver was achieved in all patients. The procedure was performed only one time in all patients (Figure 1).

Complications

No complications such as hemorrhage, hematoma, perforation, fever, pain, infection, acute liver failure or hepatic encephalopathy were observed during or after the procedure. Neither PV thrombosis nor liver neoplastic lesions was detected in any patient during the 12-mo follow-up period of abdominal ultrasonography. No patient died during the follow-up period.

Clinical outcomes

All patients survived during the 12-mo follow-up and exhibited an improvement in their clinical symptoms. Moreover, no patient experienced gastrointestinal bleeding during the follow-up period.

The serum albumin (ALB) level increased in the early postoperative period compared with that before the procedure, and reached a maximum at 6 mo (Figure 2A). In the 12^{th} mo, the serum ALB level decreased slightly but was still within the normal range. The serum ALB levels in the first month and third month after the procedure were higher than at the baseline levels (35.76 g/L ± 5.87 g/L *vs* 27.58 g/L ± 4.91 g/L, $P < 0.01$; 34.64 g/L ± 4.10 g/L *vs* 27.58 g/L ± 4.91 g/L, $P < 0.05$).

Generally, the grade of ascites detected by abdominal ultrasonography was reduced in the early postoperative period and continued to decrease during the first 6 mo after

the procedure. However, there was a slight increase in size in the 12th mo, but still smaller than at baseline (Figure 2B). However, the changes were not statistically significant at each follow-up time point compared with that before treatment.

The trend of Child-Pugh scores was similar to that of ascites (Figure 3A). Specifically, there were four patients with a Child-Pugh class A score, one patient with a class B score, and no patient with a class C score at the sixth month after treatment, however, there were 1, 3, and 1 patient with a class A, B, and C score at baseline, respectively (Figure 3B).

DISCUSSION

The technology of EUS-FNI uncovers a novel pathway for stem cell infusion for the treatment of DLC. This study demonstrated that the use of EUS-FNI for intraportal delivery of stem cells was feasible, safe and could alleviate severity in patients with DLC.

In this study, we used EUS-FNI to directly transfuse the autologous bone marrow into the PV. Traditionally, stem cell therapy is administered through the peripheral vein[13], the hepatic artery under fluoroscopic guidance[14], and the PV under the guidance of abdominal ultrasound[15]. These approaches are effective, but not without limitations. The peripheral vein method has the limitations of poor targetability as well as high risk of side effects, including the tumorigenesis of normal organs. Compared to the hepatic artery, blood flow through the PV has a larger volume and slower velocity, which is more conducive for the implantation of stem cells. In addition, EUS prevents radiation exposure associated with fluoroscopy. Compared with abdominal ultrasound, EUS has advantages of improved visualization of blood vessels within and around the liver with less interference by ascites, bowel gas or abdominal wall fat. Other potential benefits include increased efficiency and convenience to patients who require concurrent esophagogastroduodenoscopy and EUS.

Although injury to adjacent vascular structures can be avoided using real-time Doppler, the risk of bleeding during EUS-FNI significantly determines the safety of

operation, especially when performing FNI within the PV. The use of an optimally sized puncture needle in FNI can significantly reduce tissue injury and bleeding. Magno *et al*[16] investigated the differences in 19G, 22G, 25G FNA needles for EUS-guided angiography in a live porcine model. The results revealed that the 25G FNA needle did not bring about any visible vascular injury or bleeding. The 22G needle left a visible puncture mark on the vessels without any active bleeding, while the 19-gauge needle caused a localized vascular hematoma around the large-caliber vessels. However, smaller-caliber needles generated higher resistance to injection of the iodinated contrast. For this reason, the 22G FNA needle was selected to puncture the portal vein under the guidance of EUS. Moreover, a larger gauge needle size allows adequate flow of fresh bone marrow to minimize the time within the needle, and appears to reduce clotting of bone marrow compared with smaller gauge needles[17]. In our study, all patients were successfully treated with fresh autologous bone marrow injected into the PV with a 22G FNA-needle under the guidance of EUS. No bleeding-related complications, such as hemorrhage or hematoma, were detected by Doppler. Moreover, no patient developed portal vein thrombosis during the 12-mo follow-up period. These results indicated that EUS-guided intraportal FNI using a 22G FNA needle can be a safe approach for bone marrow infusion.

In addition, the transgastric and transhepatic approach was chosen for the advancement of the needle as it was assumed to be safer than the transduodenal approach. This approach provided a natural tamponade of the needle track by the surrounding liver parenchyma during withdrawal and thereby preventing post-procedural bleeding[18]. Accordingly, color Doppler detected no bleeding within the needle track after the removal of needle in the current study. Furthermore, no complications such as perforation, infection, impaired liver function or PV thrombosis were detected during the follow-up, suggesting that this operation was safe.

Drug delivery by EUS-guided intraportal FNI offers an accurate targetability. The concentration of drug within the liver is augmented while drug concentration in the peripheral circulation is reduced, which can increase the efficacy and reduce the side

effects of stem cell therapy. Faigel *et al*[19] performed EUS-guided portal injection chemotherapy (EPIC) for treatment of hepatic metastases in a porcine model. In their study, pigs were treated with irinotecan, doxorubicin, or ALB-bound paclitaxel nanoparticles by either EPIC or systemic injection. In their research, drug delivery by EPIC showed a higher hepatic concentration and a reduction in both systemic and cardiac levels compared to that by injection in systemic circulation. Owing to superior targetability, the volume of bone marrow used in our study was less and the clinical outcomes, especially serum ALB, ascites and Child-Pugh score, were almost equally beneficial compared to systemic injection in a prior study[13].

There are several limitations of this study. This study was a single center, single arm clinical study. The sample size was small and there was no control group. Moreover, the follow-up period was short. Future multicenter and larger controlled studies with longer follow-up periods are required to determine the real potential of our novel technique for the treatment of DLC. Besides, we did not obtain the evidence of homing of the transplanted bone marrow in liver, and we will design animal experiment to further prove this hypothesis.

Despite these limitations, the application of EUS-FNI for intraportal stem cell therapy can be combined with the EUS-guided intervention with coils and cyanoacrylate glue in the treatment of both DLC and gastric varices, which is one of its most common complications[20]. Most of all, a comprehensive endoscopic evaluation and therapy of patients with DLC by a gastroenterologist will be practical. In these cases, variceal screening, EUS elastography, EUS-guided portal pressure gradient measurement[21], EUS-guided liver biopsy[22], and EUS-FNI for treatment of varices and DLC may all be conducted in the same endoscopic session.

CONCLUSION

In conclusion, this study demonstrated that the use of EUS-guided FNI for intraportal delivery of bone marrow was feasible, safe and appeared effective in patients with DLC.

This treatment may be a safe, effective, non-radioactive, and minimally invasive treatment for DLC.

Figure 1 Endoscopic ultrasonography-guided intraportal fine needle injection of autologous bone marrow.

Figure 2 The changes in patients who underwent endoscopic ultrasonography-guided autologous bone marrow infusion. A: Serum albumin; B: The depth of ascites was evaluated by abdominal ultrasound. ALB: Albumin. $^{a}P < 0.05$, $^{b}P < 0.01$.

Figure 3 The changes in patients who underwent endoscopic ultrasonography-guided autologous bone marrow infusion. A: Child-Pugh scores; B: Classes.

Table 1 Baseline characteristics of patients

Item	P1	P2	P3	P4	P5
Age (yr)	30	71	136	57	54
HGB (g/L)	63.00	107.00	3.91	81.00	115.00
RBC (× 10^{12}/L)	2.15	3.05	28.00	2.74	4.22
PLT (× 10^{9}/L)	61	92	2.18	78	115
WBC (× 10^{9}/L)	2.80	3.34	41.00	2.81	2.07
ALT (U/L)	26	46	52	29	27
AST (U/L)	43	82	38	52	36
TBIL (μmol/L)	28.3	18.3	27.2	10.9	14.9
ALB (g/L)	26.2	23.5	16.0	25.0	36.0
PT (s)	18.0	13.3	40.3	16.0	16.8
APTT (s)	48.6	37.7	23.4	40.5	31.7
TT (s)	20.00	19.50	0.99	20.60	17.90
FIB (g/L)	1.48	1.60	405.76	1.64	1.95
HA (ng/mL)	124.35	674.70	204.40	297.91	123.04
LN (ng/mL)	41.15	33.83	67.56	73.10	25.86
IV-C (ng/mL)	77.16	122.50	81.95	57.27	25.47
PCⅢ (ng/mL)	74.20	125.50	14.00	58.48	28.56
Ascites (mm)	101	80	11	86	18
PV (mm)	11.0	10.0	24.8	10.0	16.0
LS (KPA)	37.9	29.8	283.0	18.0	20.2
FAP (db/m)	235	220	None	246	243
PVT	None	None	None	None	None
Neoplastic lesions	None	None	9	None	None
Child-Pugh score	10	9	136	9	6

IV-C: Collagen IV; ALB: Albumin; ALT: Alanine aminotransferase; APTT: Activated partial thromboplastin time; AST: Aspartate aminotransferase; FAP: Fat attenuation parameters; FIB: Fibrinogen; HA: Hyaluronic acid; HGB: Hemoglobin; LN: Laminin; LS: Liver stiffness; PC III: Procollagen III; PLT: Platelet; PT: Prothrombin time; PV: Portal vein; PVT: Portal vein thrombosis; RBC: Red blood cell; TBIL: Total bilirubin; TT: Thrombin time; WBC: White blood cell.

80887_Auto_Edited-Check.docx

ORIGINALITY REPORT

15%
SIMILARITY INDEX

PRIMARY SOURCES

1	Priscilla Magno, Chung-Wang Ko, Jonathan M. Buscaglia, Samuel A. Giday et al. "EUS-guided angiography: a novel approach to diagnostic and therapeutic interventions in the vascular system", Gastrointestinal Endoscopy, 2007 Crossref	94 words — 3%
2	www.ncbi.nlm.nih.gov Internet	60 words — 2%
3	Hosny Salama, Abdel-Rahman N Zekri, Eman Medhat, Shereen A Al Alim et al. "Peripheral vein infusion of autologous mesenchymal stem cells in Egyptian HCV-positive patients with end-stage liver disease", Springer Nature, 2014 Internet	59 words — 2%
4	"The 21st Conference of the Asian Pacific Association for the Study of the Liver", Hepatology International, 2011 Crossref	37 words — 1%
5	Rajat Garg, Tarun Rustagi. "Endoscopic Ultrasound-guided Portal Venous Access:", Journal of Clinical Gastroenterology, 2017 Crossref	37 words — 1%
6	bsdwebstorage.blob.core.windows.net Internet	35 words — 1%

7	Jason Y. Huang, Jason B. Samarasena, Takeshi Tsujino, John Lee, Ke-Qin Hu, Christine E. McLaren, Wen-Pin Chen, Kenneth J. Chang. "EUS-guided portal pressure gradient measurement with a simple novel device: a human pilot study", Gastrointestinal Endoscopy, 2017 Crossref	33 words —	1%
8	www.wjgnet.com Internet	28 words —	1%
9	Renren Wang, Xiaoli Huang, Tao Zhou, Yueyue Li, Mengmeng Ding, Huawei Xu, Yanjing Gao. "Safety and feasibility of early oral nutrition after endoscopic treatment for patients with liver cirrhosis: A historical prospective and comparative effectiveness study", Journal of Parenteral and Enteral Nutrition, 2022 Crossref	15 words —	< 1%
10	link.springer.com Internet	14 words —	< 1%
11	journals.plos.org Internet	13 words —	< 1%
12	Carmelo Sidoti, Umberto Agrillo. "Chronic Cortical Stimulation for Amyotropic Lateral Sclerosis: A Report of Four Consecutive Operated Cases after a 2-Year Follow-up: Technical Case Report", Neurosurgery, 2006 Crossref	11 words —	< 1%
13	upload.umin.ac.jp Internet	11 words —	< 1%
14	atm.amegroups.com Internet	10 words —	< 1%

15	prostate.uroonco.uroweb.org Internet	10 words — < 1%
16	Herberger, Rustenbach, Haartje, Blome, Franzke, Schäfer, Radtke, Augustin. "Quality of life and satisfaction of patients with leg ulcers - results of a community-based study", Vasa, 2011 Crossref	9 words — < 1%
17	Schickwann Tsai. "Isolation of a human stromal cell strain secreting hemopoietic growth factors", Journal of Cellular Physiology, 04/1986 Crossref	9 words — < 1%
18	clinicaltrials.gov Internet	9 words — < 1%
19	covid-19.cochrane.org Internet	9 words — < 1%
20	repub.eur.nl Internet	9 words — < 1%
21	www.em-consulte.com Internet	9 words — < 1%
22	journaltcm.com Internet	8 words — < 1%
23	static.frontierspartnerships.org Internet	8 words — < 1%
24	"Endoscopy", The American Journal of Gastroenterology, 2015. Crossref	7 words — < 1%

EXCLUDE QUOTES	OFF	EXCLUDE SOURCES	OFF
EXCLUDE BIBLIOGRAPHY	OFF	EXCLUDE MATCHES	OFF