

CASE REPORT

# Mesenchymal stem cell therapy for advanced liver cirrhosis: A case report

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**Abstract**

Mesenchymal stem cell (MSC) transplant may offer an alternative to liver transplantation in patients with end-stage liver disease. However, its efficacy remains uncertain. MSC was performed on a 50-year-old male with decompensated (Child-Turcotte-Pugh grade C) alcoholic liver cirrhosis due to an absence of donors for adult-deceased and living-related liver transplantation. Autologous bone marrow-derived MSCs were harvested from the patient and cultured using standard protocols. The MSCs were subsequently re-administrated into the liver via hepatic intra-arterial infusion on two separate occasions. After infusion, there was an improvement in biochemical parameters (serum total bilirubin, serum albumin), and a reduction of diuretic use for ascites for up to 8 weeks. However, all biochemical and clinical parameters deteriorated on long-term follow-up without any further infusions. The patient eventually succumbed to his disease. MSC transplantation may have a clinical benefit on adult patients with end-stage liver cirrhosis, but this appears to be transitory.

**Introduction**

Decompensated cirrhosis is a terminal condition, with a median survival of 2 years.<sup>1</sup> Liver transplantation is currently the only definitive therapy for decompensated cirrhosis. However, due to shortcomings such as scarcity of organ donation, high-operational cost, lack of expertise, and centers that offer liver transplantation services, there is an urgent need to develop alternative therapies.<sup>2</sup> Stem cell infusion therapy is one such alternative therapy. There are two main sources of stem cells: embryonic and non-embryonic source, commonly termed as adult or somatic stem cells. Under specific environment, adult stem cells could be cultured to produce various types of cell lineage, also known as induced pluripotent stem cell.<sup>3</sup>

Adult stem cells are mainly derived from bone marrow (BM) and other organs such as the heart, brain, or liver. Stem cells from the BM give rise to hematopoietic stem cells (HSC) as well as stromal or mesenchymal stem cells (MSC).<sup>1</sup> Both HSC and MSC therapies have been explored in an experimental manner for both acute and chronic liver injuries, with human BM MSC showing some promise in recent reports.<sup>2,4,5</sup>

Due to a lack of liver transplantation services in our country, MSC transplantation therapy was administered in a single case of decompensated liver cirrhosis in our center, at the request and personal cost of the patient. This provided us an opportunity to examine its clinical effects and benefits.

**Case report**

A 50-year-old male, of Indian ethnicity, had initially presented to this institution with features of decompensated chronic liver disease 5 years prior. He had a history of alcohol excess, but no addiction, and a negative result for all other etiological parameters for liver disease. At initial presentation, he had Child-Turcotte-Pugh (CTP) grade C disease, with gross ascites, and peripheral edema, but no overt jaundice. His symptoms and signs initially responded to alcohol abstinence, diuretics (Spironolactone and Frusemide), and a branch-chain amino acid supplemented diet. However, the doses of diuretics continued to increase over the ensuing years, despite abstinence of alcohol by the patient. Four years after initial presentation, he developed two episodes of hepatic encephalopathy requiring hospitalization. He was counseled about liver transplantation abroad (due to a lack of cadaveric and living-related liver transplantation services locally), but no willing donor could be found. At the patient's insistence, we considered the option of MSC transplantation in view of his deteriorating clinical condition.

Following institutional ethical approval and informed consent from the patient, an autologous MSC transplantation was conducted in 2014 on two separate occasions. An initial harvesting involved aspirating 100 ml of BM fluid from the posterior iliac crest under local anesthesia. The aspirated BM was transferred into anticoagulant solution (acid citrate dextrose),

transported in cold chain, and immediately processed under a controlled environment in a cGMP certified facility. Ficoll gradient centrifugation procedure was carried out to harvest the BM Mononuclear cells (BM-MNC) by isolating the buffy coat.

Approximately  $60\,000/\text{cm}^2$  of BM-MNCs was plated in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) (GIBCO-USA), supplemented with 10% fetal calf serum (GIBCO-USA), and 1% penicillin/streptomycin (GIBCO-USA). The cultures were maintained at  $37^\circ\text{C}$  in a 5%  $\text{CO}_2$  atmosphere. Non-adherent cells were removed on day 3 and the adherent cells were cultured until 70–80% confluence and harvested by 0.25% trypsin/1 mM EDTA (GIBCO-USA). Cell viability was evaluated by Trypan blue exclusion dye method. Harvested cells were serially subcultured ( $6000/\text{cm}^2$ ) until passage IV, then washed with phosphate-buffered saline, and final cell pellet was resuspended with injectable saline solution in the ratio of  $2 \times 10^6/\text{kg}$  body weight. Sterility test, using bacterial and fungal agar plates, and an endotoxin test using Limulus amoebocyte lysate (Wako, Tokyo, Japan), immunophenotyping and karyotyping were carried out to confirm the asepsis of the products.

Flowcytometry (FC-500; Beckman Coulter, CA, USA) analysis was performed by using MSCs' specific antibodies: CD73-PE, CD90-FITC, CD44-PE, CD105-PerCP-Cy (BD Biosciences). For 20 GTL-banding (G bands using trypsin and Leishman's stain), passage IV cells were sent for karyotyping analysis to the Cytogenetics Laboratory, National University Hospital, Singapore.

MSC transplantation was performed via intrahepatic arterial infusion. A total of 120 million MSC stem cells were infused during the first infusion. A second transplantation was repeated 40 days later, whereby a further number of 120 million MSC stem cells were infused into the patient.

The patient was monitored on an outpatient basis following the first and second MSC transplantations. Routine

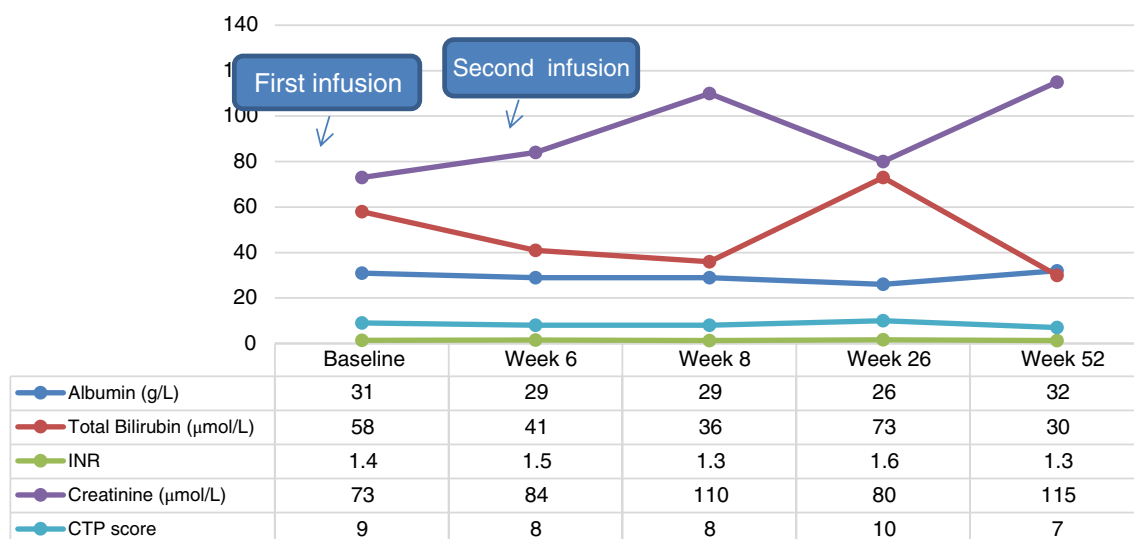
biochemical parameters requirement of diuretics for ascites and the overall CTP score were assessed prospectively. For a period of up to 8 weeks after initial MSC transplantation, there appeared to be an improvement in serum bilirubin international normalised ratio (INR) and even the CTP (Fig. 1). Furthermore, a reduction in the daily dosage of Spironolactone from 100 mg to 25 mg was possible without deterioration in the amount of abdominal ascites. However, after 8 weeks, the serum bilirubin INR and CTP appeared to deteriorate. The serum creatinine increased transiently after MSC infusion, but began to improve after 8 weeks.

Due to lack of financial funds, we were unable to perform further MSC transplantations for the patient, but he continued to be monitored in the general outpatient clinics. His condition gradually deteriorated and he eventually succumbed to his illness during an inpatient admission 12 months after his last MSC transplantation.

## Discussion

Stem cells contribute to liver regeneration via several mechanisms. The hypothesized mechanisms are transdifferentiation into hepatocytes, cell fusion of stem cells and diseased hepatocytes, division of fused cells to repopulate the liver as well as enhancement of angiogenesis which supports the rapid expansion of healthy hepatocytes.<sup>1,2,4–6</sup>

Autologous transplantation refers to transplantation of stem cells derived from one's own body whereas allogenic transplantation refers to transplantation of stem cells which were derived from a human leucocyte antigen (HLA)-matched sibling or unrelated donor (from a stem cell bank). These two methods have their own advantages and disadvantages. Comparatively, autologous transplant has no risk of graft *versus* host disease (GVHD) and transmissible infection, is far more commonly used in advanced liver cirrhosis patients but has a longer waiting time (to obtain, harvest, and culture stem cells), limited quantity of



**Figure 1** Biochemical parameters and Child-Turcotte-Pugh (CTP) scores before and after MSC infusion in the patient with advanced cirrhosis. —●—, Albumin (g/L); —●—, total bilirubin (µmol/L); —●—, INR; —●—, creatinine (µmol/L); —●—, CTP score.

stem cells and theoretical risk of “lesser quality” stem cells due to influence of primary disease. For our patient, we decided to proceed with autologous MSC transplantation for the reasons mentioned above, and a lack of HLA-matched sibling donor.

There are several routes to administer MSC, with direct infusion into hepatic artery or portal vein being the preferred route compared to peripheral infusion. This is due to the fact that peripheral infusion of MSC results in dissipation in various parts of the human body before finally being deposited in the liver. As a result, although simpler to perform, peripheral infusion of MSC leads to a lower quantity of MSC in the liver compared to direct route administration. However, the direct route infusion methods are not without their disadvantages. They have a higher cost, can lead to hepatorenal syndrome with usage of contrast agents, and have a risk of worsening portal hypertension when the portal vein route is used.

Several studies which involve autologous BM infusions for advanced liver disease patients have demonstrated an improvement in the liver’s synthetic function, mainly an increase in serum albumin level, a reduction in INR, total bilirubin, CTP, and model of end-stage liver disease score.<sup>6–11</sup> Some of these studies specifically look into patients with chronic hepatitis B<sup>11,12</sup> and C<sup>6,13</sup> related to liver cirrhosis, with similar encouraging outcomes. However, all these studies report only short-term improvement of liver function at 1–6 months after therapy with decline of function back to baseline on longer follow up (12 months or longer). Amin *et al.* reported improvement in clinical features such as improvement in ascites, pedal edema, and encephalopathy.<sup>6,11</sup> A recent systematic review examining the effect of MSC transplantation in cirrhosis reported that 17 of 25 studies were able to demonstrate significant improvements in liver function tests.<sup>1</sup> However, there were no studies demonstrating a survival benefit following MSC transplantation.

Our experience of this single case of MSC transplantation in end-stage liver cirrhosis concurs with the published literature. We were able to demonstrate some biochemical and clinical improvements in the patient following two infusions of MSC, but the benefits appeared to be short-lived. However, no immunological marker was assessed and may be a limitation in this case. Further infusions could not be performed due to financial restrictions, but they may have led to an improvement in the patient’s quality of life.

In conclusion, our single case experience suggests that autologous MSC transplantation via intrahepatic arterial infusion for advanced cirrhosis is safe. However, it only has a short-term clinical

benefit. MSC infusion may be considered as a bridging therapy for advanced liver cirrhosis, while awaiting liver transplantation.

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