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Preliminary Study on Intrasplenic Implantation of Artificial Cell Bioencapsulated Stem Cells to Increase the Survival of 90% Hepatectomized Rats

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Abstract

We implanted artificial cell bioencapsulated bone marrow mesenchymal stem cells into the spleens of 90% hepatectomized (PH) rats. The resulting 14 days survival rate was 91%. This is compared to a survival rate of 21% in 90% hepatectomized rats and 25% for those receiving free MSCs transplanted the same way. Unlike free MSCs, the bioencapsulated MSCs are retained in the spleens and their hepatotrophic factors can continue to drain directly into the liver without dilution resulting in improved hepatic regeneration. In addition, with time the transdifferentiation of MSCs into hepatocyte-like cells in the spleen renders the spleen as a ectopic liver support.

Keywords

mesenchymal stem cells; bone marrow; liver failure; liver regeneration; partial hepatectomy; regenerative medicine; transplantation; spleen; microencapsulation: intrasplenic implantation; artificial cells

INTRODUCTION

The therapeutic potential of mesenchymal stem cells (MSCs) derived from bone marrow includes their ability to differentiate into different cell lineages and their secretion of cytokines and growth factors. There are mainly two types of stem cells in bone marrow, hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). MSCs can secrete factors like interleukin 6 (IL-6) and Hepatotrophic factor HGF [1,2] that stimulates liver regeneration. We reported earlier that intraperitoneal implantation of bioencapsulated bone marrow nucleated cells into 90% hepatectomized rats significantly increased their survival rate [3]. There was no significant increase in survival rates for those receiving free MSCs transplanted intraperitoneally. We showed that bioencapsulated MSCs are retained in the peritoneal cavity and their hepatotrophic factors can drain directly into the portal circulation to the liver without dilution, resulting in improved hepatic regeneration. Free MSCs implanted intraperitoneal rapidly escaped from the peritoneal cavity and therefore did not have a significant effect on increasing the survival.

Potential problems of intraperitoneal implantation of artificial cells bioencapsulated stem cells including peritonitis and foreign body reaction resulting in fibrosis may have an adverse effect on the intestine. In the present study we implanted artificial cells bioencapsulated MBCs into the spleens of 90% hepatectomized rats. The resulting 14 days survival rate was 91%. This is compared to a survival rate of 21% in 90% hepatectomized rats. The survival rates of those receiving free MSCs transplanted intraperitoneally was 25%.

MATERIALS AND METHODS

Animals

Male Wistar rats, 200–225 g, purchased from Charles River (St. Constant, Canada) were donors for bone marrow cells. Syngeneic male Wistar rats were used as the recipients.

Bone Marrow Stem Cells Isolation and MSCs Expansion

The details have been reported elsewhere [3]. Briefly, Wistar rats were anaesthetized with sodium pentobarbital and both femurs were isolated. Serum-free L-DMEM (low glucose DMEM, GIBCO, BRL) was used to flush out bone marrow cells from the femurs using a 5 ml syringe with a 22 gauge needle. Bone marrow mononuclear cells were isolated with Percoll gradient density centrifugation. Cells were resuspended in expansion medium (DMEM low glucose, 10% FBS, 2 mM L-glutamine, HEPES, 100 U/ml penicillin and 100 µg/ml streptomycin, Amphotericin B 2.5 µg/ml, 10 ng/mL epidermal growth factor (EGF), 10 ng/mL bFGF) and seeded in 10 cm culture dishes at a density of 5×10^4 cells/cm², incubated in 95% air, 5% CO₂ at 37°C, with fresh medium change every 3–4 days. The adherent cells were allowed to reach 80% confluence, then were passaged. Usually after 3 passages, the cells are purified as spindle-shaped MSCs, and could be harvested for further experiment use.

Microencapsulation of MSCs

Alginate polylysine alginate (APA) microencapsulation method was used to encapsulate the MSCs as described previously [4]. Briefly, MSCs 2×10^8 were suspended in 15 ml 1.5% sodium alginate solution (Inotech, Rockville, MD, USA). The cell suspension was extruded through droplet generator (NISCO Encapsulator, NISCO Engineering AG, Switzerland). The beads formed were allowed to fall into a PYREX dish containing 100 mM CaCl₂. After the beads were allowed to gel in the calcium solution for 5 min, the beads were immersed in 50 mg% poly-L-lysine solution for 15 min., washed with buffered saline (0.85% NaCl, 10mM HEPES, 20mM D-fructose, PH7.4), and then immersed into 0.2% sodium alginate for 10 min. Finally, the beads were placed in 50 mM sodium citrate for 20–30 min to dissolve the inner alginate gel and to form an APA membrane. The final microcapsules containing MSCs were incubated in L-DMEM without supplements, serum-free, in 5% CO₂, 37°C incubator for 4 hours prior to transplantation.

Ninety Percent PH and Protocols

The 90% PH was performed by removing the median, left lateral lobes, and right upper and lower lobes, leaving only the caudate lobe [3]. For the sham operation, we only carried out abdomen incision and cutting of the suspending ligament of the liver, and then closed the

incision. Forty-eight animals were randomly divided into 4 groups and were administered the respective agents, sham control (n = 10); 90% PH control (n = 14); 90% PH intrasplenic transplanted with 3×10^7 microencapsulated MSCs (n = 12); 90% PH intrasplenic transplanted with 3×10^7 free MSCs (n = 12).

Immediately after 90% PH, MSCs microcapsules were injected intrasplenically using an 18 gauge needle, and 23G needle for free MSCs injection. Injections were made through 3–4 sites in spleen. Absorbable hemostat (Surgicel NU-KNIT, Ethicon Inc., NJ, USA) was used to prevent bleeding after injection.

Statistical Analysis

Kaplan-Meier survival curve was used to analyze the survival, χ^2 (Chi-Square) method was used for animal survival comparison. A *p*-value of less than 0.05 was considered significant.

RESULTS

Survival analysis showed that 2-week survival after transplantation differed significantly between the microencapsulated MSCs group and the 90% PH group ($p < 0.01$, Table 1), whereas there was no difference between free MSCs group and the 90% PH group ($p > 0.05$, Table 1). The liver to body weight ratio shows that livers have regenerated to normal weight for those that survived for 14 days.

DISCUSSION

Intrasplenic transplantation of microencapsulated MSCs significantly prolongs survival of 90% PH rats, whereas this effect was not observed in the free MSCs transplantation. The initial effect is the secretion of hepatotrophic factors that drain into the liver to increase liver regeneration. In addition, with time the MSCs transdifferentiate into hepatocyte-like cells in the spleen may render spleen as an ectopic liver support. Further studies being carried out include the mechanism of growth factors and cytokines involved in this process, and the long-term fate of microencapsulated MSCs in spleen, and their liver support capacity. Intrasplenic implantation of artificial cells bioencapsulated cells is also a new approach for cell/stem cell therapy.

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Table 1

Survival of 90% PH rats with intrasplenic MSCs transplantation

Day	Sham (n = 10) (%)	PH (n = 14) (%)	PH + encapMSC (n = 12) (%)	PH + free MSC (n = 12) (%)
0	10/10 (100)	14/14 (100)	12/12 (100)	12/12 (100)
1	10/10 (100)	12/14 (85.7)	12/12 (100)	12/12 (100)
2	10/10 (100)	8/14 (57.1)	11/12 (91.6) *	7/12 (58.3)
3	10/10 (100)	3/14 (21.4)	11/12 (91.6) **	4/12 (33.3)
4	10/10 (100)	3/14 (21.4)	11/12 (91.6) **	4/12 (33.3)
14	10/10 (100)	3/14 (21.4)	11/12 (91.6) **	3/12 (25)

*
p <0.05;**
p <0.01; versus the 90% PH model group