Availability of a Magnetic Method for Hepatocyte Transplantation

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ABSTRACT

Introduction. Hepatocyte transplantation is a promising alternate for the treatment of hepatic diseases. Hypothermic preservation of isolated human hepatocytes is potentially a simple and convenient strategy to provide on-demand hepatocytes in the quantity sufficient and the quality required for biotherapy. Isolated fresh hepatocytes include damaged cells that are also early apoptotic cells, which is not ideal for hepatocyte transplantation. However, this does not reflect cell viability, although it is considered that it adversely affects cell survival after transplantation. We aimed to harvest these hepatocytes and filter the apoptotic cells using a magnetic method to provide a transplantation source.

Materials and Methods. Rat hepatocytes were isolated from caudate lobes using manual enzymatic perfusion. The hepatocyte yield was $5.3 \pm 0.66 \times 10^9$ cells/g of liver tissue, with a viability of $82.3 \pm 3.5\%$. Two samples of hepatocytes were freshly isolated, one using the magnetic method, and the other without. The magnetic method was performed using DynaMag-15 Magnet, and Annexin V Antibody was used on the early apoptotic cells. We evaluated the viability and plate efficiency of the cells after 24 hours at 37°C. Hepatocytes were isolated using cell separation method, and 30×10^6 cells were mixed with 1.0 mL of Dulbecco's Modified Eagle's Medium (DMEM) and directly injected into the spleen of Lewis rats (150–250 g) using 24-gauge needles. Blood samples were collected on days 0, 3, 7, and 14, and the blood albumin level was measured using enzyme-linked immunosorbent assay (ELISA):G1, control (medium injection); G2, fresh hepatocyte transplant using the magnetic method; and G3, fresh hepatocyte transplant without the magnetic method.

Results. The viability was $84.9 \pm 2\%$ for fresh hepatocytes and $80.7 \pm 1.2\%$ for hepatocytes isolated using the magnetic method. The magnetic method does not damage the cells ($73.5 \pm 2\%$ vs $35.2 \pm 2\%$ after 24 hours), preserving hepatocyte. The albumin level accepted significantly increased in the magnet-treated group compared with the nonmagnet group. Simultaneously, the spleen in which these hepatocytes were transplanted could be used to observe the hepatocytes; the cells were transplanted 14 days later, and the magnet-treated group had significantly higher levels of hepatocytes than the nonmagnet group.

Conclusion. We developed an effective technique for hepatocyte isolation for short-term preservation. As a result, we believe that transplantation not only improves the cell transplantation effect but also allows the cells to be stored efficiently using the magnetic method. These results demonstrate the usefulness of hepatocyte hypothermic preservation for cell transplantation.

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LIVER transplantation improves the survival and quality of life of patients with life-threatening liver diseases. However, considering its technical complexity, this procedure is associated with significant morbidity and mortality. Experimental animal studies have indicated that the transplantation of isolated hepatocytes can be a useful technique for treating patients with liver-based metabolic diseases and liver failure [3,5,6,9–13,21]. Because hepatocytes can be transplanted using minimally invasive percutaneous techniques, this relatively low-risk procedure can be applied to patients who are not candidates for whole-organ transplantation. Unfortunately, the number of donor livers available for human hepatocyte isolation is limited by the demands for these organs in the field of whole-organ transplantation. Further, isolated fresh hepatocytes include damaged cells that are also apoptotic cells, which is not ideal for hepatocyte transplantation [1,2,7,8]. However, this does not reflect the viability of the cells, and it is believed that transplantation of apoptotic cells adversely affects cell survival after transplantation [14,15,16].

We aimed to harvest these hepatocytes and filter the apoptotic cells using a new magnetic method to provide a source for transplantation.

MATERIALS AND METHODS

Animals and Chemicals

Inbred male Lewis rats (150–250 g) and Nagase analbuminemic rats (NARs; 100–250 g) were obtained from Chubu Kagaku Shizai (Nagoya, Japan) and maintained at the Animal Resource Facility of Fujita Health University. The animals were maintained on standard laboratory chow and a 12-hour light/dark cycle. All procedures performed on the rats were approved by the Fujita Health University Institutional Animal Care and Use Committee and were in accordance with the Guidelines for the Humane Care of Laboratory Animals.

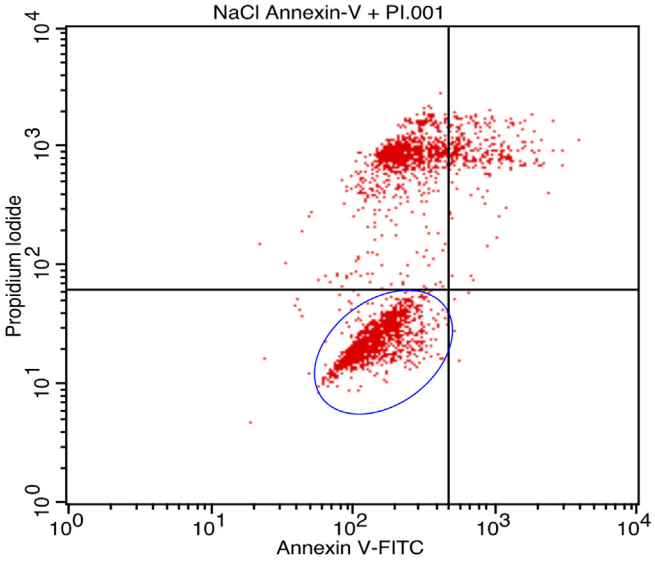
Isolation of Lewis Rat Hepatocytes

Hepatocytes were isolated from Lewis rats using a two-step in situ collagenase (Liberase Research Grade; Roche, Basel, Switzerland) perfusion technique that was introduced by Berry and Friend [4] and modified by Seglen [22]. The isolated cell viability consistently remained between 85% and 90%, as determined using trypan blue (Sigma–Aldrich Co., LLC, Tokyo, Japan) exclusion. However, the cell viability was only 54.3% using fluorescence-activated cell sorting (annexin V and propidium iodide [PI]), although it was 85% using trypan blue (Fig 1) [23–27].

This result pointed out that isolated fresh hepatocytes included damaged cells that also are early apoptotic cells.

Magnetic Method

The magnetic method was tested using a DynaMag-15 Magnet, and Annexin V Antibody was used on the early apoptotic cells. This magnet can be rotated 360°, thus allowing the user the flexibility to tilt the magnet for better visual control and optimal ergonomic handling. Tilting the magnet during separation allows the user to collect any bead-bound cells that might be left in the tube lid. Once tilted, the magnet remains in position until adjusted. To increase pipetting efficiency, we recommend slowly turning the tubes back



Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	4852	35.50	19.41	257.41	241.27	839.68	740.69
UR	1356	9.92	5.42	894.67	810.07	996.87	908.48
LL	7427	54.35	29.71	159.29	147.96	24.59	22.97
LR	31	0.23	0.12	624.47	610.64	33.76	31.26

Fig 1. Annexin V-fluorescein isothiocyanate and PI, the viability was only 54.3% using fluorescence-activated cell sorting (Annexin V and PI) even 85% using trypan blue.